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I. Agriculture

Abstracts Nos. 1—43

1. **Studies on the morphogenesis of crop plants under different growing conditions. II. The formation of lateral buds in rice and wheat plants.** Koou YAMAZAKI (Proc. Crop Sci. Soc. Jap. 28, 262–265, 1960)

These experiments were carried out to examine the formation of lateral buds in rice and wheat plants, and also to find its relationships to the leaf development on the main stem.

In rice plants, when the base of a leaf margin grows as far as the opposite side of the shoot apex, the lateral bud primordium first appears at its outer position. The primordium is recognized by the repeated periclinal divisions of the inner cells. The bud primordium thus formed remains in the same state until the adjacent upper leaf begins to emerge from the adjacent lower leaf sheath, when the bud primordium suddenly grows up and forms a prophyll.

In wheat plants, the same phenomena were observed, in spite of the conspicuous changes in the number of leaf primordia involved in the apical bud.

All these phenomena were found in every leaf base observed except in the first and the second leaf bases of rice plants. At the first leaf base, the lateral bud primordium is first observed, but it does not grow any more and degenerates. At the second leaf base, the primordium develops, but sometimes it fails to form a prophyll and becomes a parenchymatous protuberance.

In rice plants, neither the amounts of nitrogen fertilizer applied nor the plant spacings were effective on the formation of lateral buds mentioned above.

2. **Physiological studies on the vernalization of wheat plants. II. The progress of vernalization under the field condition.** Aiya ISHIHARA (Proc. Crop Sci. Soc. Jap. 28, 316–320, 1960)

The experiments were carried out to follow the progress of vernalization in winter wheat plants, var. Norin No. 64, under the field condition in late autumn and winter seasons. At successive stages, some of the growing plants in field were removed to the continuous light condition under the temperature of about 20°C, and the affected degree by natural vernalization in the plants were estimated by counting the number of leaves newly formed before flowering in the main stem, subtracting the number of determinate leaves at the time of the removing from the final leaf number.

It was found that the vernalization progressed almost to the completion during one or one and a half month from sowing time. The results indicated that the vernalization under the field condition proceeded with a similar rate as that of seeds treated at 5°C, even though the temperature in the field was higher than 5°C, f.i., the daily mean temperature was 15–7°C during the vernalization period in one experiment. From the examination of shoot apex development, it was suggested that in the vernalized plants the production of leaf or bract primordium is activated, which in turn leads to the spikelet initiation.

3. **Analysis of grain production in rice plant. III. Studies of the effects of various nitrogenous nutrient levels and light conditions on the factors constituting field photosynthetic ability and dry matter productivity in paddy rice.** Tomoshiro TAKEDA and Atsuhiko KUMURA

(Proc. Crop Sci. Soc. Jap. 28, 175-178, 1959)

Paddy rice plants, the variety Norin No. 29, supplied with nitrogen at various levels were grown under various light conditions, and the effects of the nutrient on the factors constituting field photosynthetic ability, respiration and dry matter productivity of the rice plants were studied.

In their previous papers, the authors have postulated following formula for the dry matter productivity of the rice plants growing under community conditions;

$$\varDelta W = k \cdot L \cdot S \cdot {}_{Po} \cdot {}_{\rho} - Rw$$

In the present paper, the authors elucidated that the dry matter productivity of rice plants would be shown more accurately by the following formula;

$$\varDelta W = S \cdot {}_{Po} \cdot \frac{\alpha I}{1+\alpha I} \cdot D - Rw$$

where, $\varDelta W$ denotes the dry matter produced per day, S: leaf area, ${}_{Po}$: mean photosynthetic activity per unit leaf area, D : day length, α : light curve coefficient, I: mean light intensity.

4. **Analysis of grain production in rice plant. V. Analytical studies on the varietal tolerability for heavy manuring in paddy rice.** Tomoshiro TAKEDA and Atsuhiko KUMURA (Proc. Crop Sci. Soc. Jap. 28, 179-181, 1959)

Rice varieties much tolerable for heavy fertilizing with nitrogen, Fujisaka, No. 5 and Ginmasari (former early var., latter medium var.), and untolerable varieties, Obanasawa, No. 1 and Norin, No. 21 (former early var., latter medium var.) were grown under low, medium and high nitrogenous nutrient levels respectively, and the factors constituting field photosynthetic ability, respiration and dry matter productivity of those were studied.

(1) In the varieties much tolerable for heavy fertilizing with nitrogen, total leaf area grows up much greater than that of the untolerable varieties, and the time at which the maximum leaf area attains is later than that of the latter.

(2) Under the moderate and light nitrogenous conditions, the photosynthetic capacity of the individual plant of the tolerable varieties is not different from that of the untolerable ones, however, under the heavy nitrogenous conditions it is much greater than that of the latter.

(3) In every variety field photosynthetic activity decreases gradually with the increase of total leaf area. But the rate of decrease in the tolerable varieties is less than that of the untolerable ones. In contrast, in the latter the light receiving status becomes worse with the increase of total leaf area.

5. **Cytological studies of double fertilization in wheat (*Triticum aestivum* L.).** Kiyochika HOSHIKAWA (Proc. Crop Sci. Soc. Jap. 28, 142-146, 1959)

Early mid-season and late varieties of winter wheat were used in seeking the double fertilization after artificial pollination under 20°C constant condition. Ovaries were collected at later short time intervals, and their cross and longitudinal sections were made by paraffin method.

In 0.5-1 hours after pollination, tip of the pollen tube passes through the micropyle and enters into one synergid. By the reaction between pollen tube cytoplasm and synergid cytoplasm, two sperms were discharged into embryo sac cavity.

A sperm enters the ovum on the chalazal side. By 2-3 hours after pollination the spiral shaped male nucleus penetrates to the egg nucleus where fusion occurs at 4-4.5 hours after pollination. This time required is shorter than that reported by other previous workers.

Immediately after another sperm gets nearer to polar nuclei, they migrate away together

from ovum side and come in contact with antipodals. In 2-2.5 hours after pollination, the male nucleus enters into one of the polar nuclei (1st fusion: $n+n=2n$), and through the short resting stage the 2n nucleus shows the symptom of mitosis. By 5 hours after pollination the membrane of both polar nuclei disappears and the second fusion ($2n+n=3n$) occurs. Thus the primary endosperm nucleus is formed.

This fertilization process was the same for the all varieties tested.

6. **Observations on the embryo sac containing double egg apparatus in *Triticum aestivum* L.** Kiyochika HOSHIKAWA (Bot. Mag. Tokyo, 73, 109-112, 1960)

The occurrence of abnormal embryo sac with double egg apparatus, though rare, was observed in ovules of common wheat, *Triticum aestivum* L. The frequency of its occurrence is about one-three hundredth.

The embryo sac possesses two egg cells and four synergids, and polar nuclei seem to half fuse with each other before fertilization.

Regarding the process of the embryo sac formation, it is supposed that such double egg apparatus appears through the fourth nuclear division of megaspore in mycropylar nuclear group.

This embryo sac with double egg apparatus may be thought of as a new source of polyembryony.

7. **Studies on the pollen germination and pollen tube growth in relation to the fertilization in wheat.** Kiyochika HOSHIKAWA (Pro. Crop Sci. Soc. Jap. 28, 333-336, 1960)

Pollen germination and pollen tube growth in wheat at 10°C, 20°C and 30°C temperature were studied in relation to the fertilization.

At 10°C, germination percentages with the lapse of time as well as the tube growth rate were markedly retarded. At 20°C pollen showed the best germination. And at 30°C, the germination percentages were slightly inferior to those at 20°C, however, the growth rate of the tubes was more superior to that at 20°C.

The times necessary for the first appearance of empty pollen grains on the stigma were found to be two hours at 10°C, thirty minutes at 20°C and only fifteen minutes at 30°C. And that, at all temperatures these times were in accordance with the times necessary for penetration of tip of the tube into embryo sac.

These results suggested that a pollen tube concerned with the fertilization was chosen already when only a few empty pollen grains first appeared on the stigma, and a large percentage of the pollen grains remained ungerminated at that time.

8. **Influence of temperature upon the fertilization of wheat, grown in various levels of nitrogen.** Kiyochika HOSHIKAWA (Proc. Crop Sci. Soc. Jap. 28, 291-294, 1960)

The influence of various temperatures (10°C, 20°C and 30°C) and nitrogen levels (excessive-N, medium-N and low-N) upon the double fertilization of common wheat, *Triticum aestivum* L. were studied cytologically.

Through microscopic observations of about 1,000 embryo sacs, collected at several short intervals after pollination, the progressive processes of fertilization under various conditions were brought to light. According to these nine fertilization curves, the times necessary for fertilization under nine conditions were determined respectively.

Within the range of flowering temperature (10-30°C), the time necessary from pollination to the completion of double fertilization decreased in inverse proportion to the rises in

temperature, regardless of the nitrogen levels. The fertilizations in both the excessive-N and low-N levels required longer time than those in medium-N level. However, at 20°C this difference was almost insignificant.

The results obtained from the comparative studies of the progressive pace of fertilization curves under various conditions, illustrated the cause of the variation of time necessary for fertilization among nine conditions. Moreover, they have shown that the fertilization of wheat was influenced by the tested conditions more during its first half processes than in its later half processes.

9. On the antipodal cells in gramineous plants (1). Antipodal cells of *Triticum aestivum* L. Kiyochika HOSHIKAWA (Journ. Jap. Bot. 35, 180–186, 1960)

The formation of antipodal cells of *Triticum aestivum* L. (common wheat) was studied embryologically.

At first, three antipodal cells of *Polygonum* type were differentiated through the nuclear divisions, and were situated at the middle of the embryo sac.

Immediately after the second additional division of antipodal cells, one of the cells which was located at the chalazal pole showed a singularity and formed a haustorium composed of some special shaped cells. The antipodal haustorium was short-lived and degenerated after the fourth and the last additional division of other antipodal cells, and the contiguous antipodal cells also began to degenerate successively. However, antipodal cells of micropylar side increased in their sizes accompanied by the rapid enlargement of the embryo sac.

At the flowering day, antipodal complex was situated in somewhat lateral side of the embryo sac, and consisted of 14-17 large, mature cells.

The number of the antipodal cells had no relation with the varietal and nutritional differences but varied with the age of the embryo sac remarkably.

Antipodal cells contribute not only to the early stage of the endosperm development, but also to the growing of the embryo sac.

10. Effects of nitrogen supplied at the various stages of growth, on the growth and yield of onions in the sand culture. Masatoshi IWATA, Isamu MORITA and Fujio HONDA (Journ. Hort. Assoc. Jap. 28, 96–108, 1959)

Experiments were carried out to clarify the effects of nitrogen supplied at the various stages from transplanting to harvesting time, on the growth and yield of onions in the sand culture. In both 1952 and 1955, onion seedlings (Yellow Danvers sown early in September) were transplanted late in November and harvested late in next June. Results obtained were as follows.

The bulb yields of plants which were stopped nitrogen supply since middle or late in May (completing stage of top growth) were equal to, or rather exceeded those of control plants supplied with nitrogen until harvesting time. Plants which were discontinued nitrogen supply since middle or late in April (beginning stage of bulb thickening), were decreased to some degree in both top growth and bulb yield. And in the treatments in which nitrogen supply were discontinued on and before middle or late in March (beginning stage of top growth), top growth and yields were reduced remarkably, and their bulbs were round-shaped in contrast with flat-shaped in the control.

Plants which were withheld nitrogen supply until late in February or early in March, and since then supplied with nitrogen, recovered their retardation of top growth rapidly and their bulb yields made no great difference compared with those of the control. But when nitrogen supply was withheld until late in March, or late in April, bulb yields in these

treatments could not catch up with those of control until harvesting time.

The treatments of cutting off the nitrogen supply for about one month at the various stages of growth, had no effect on bulb yields at harvesting time.

In the tops of control plants, nitrogen and dry matter contents were lower until late in February, and increased from March to May, especially the increment in April was remarkable. In the bulbs, nitrogen contents increased rapidly togethr with dry matter contents from middle or late in April, and continued to increase until harvesting time.

From results obtained, it is concluded that nitrogen supply from middle or late in March to middle or late in May is the most important for growth and yield of onion, while nitrogen supply after late in May, or before early in March is not so important as that for the above-mentioned period.

11. Effect of sowing date on the harvesting date of curd in Italian broccoli varieties. Tadayoshi SUGIYAMA and Kazuhiko TAKAHASHI (Agr. & Hort. 35, 57–58, 1960)

In order to know the effect of sowing date on the harvesting date of curd in Italian broccoli, ten varieties (or strains) were planted successively on 1st of every month from April to October in 1957 to 58.

There found considerable variation in earliness of curd formation among individuals in every variety. In from May to August plantings, it took 3 to 3.5 months from sowing to harvest of top curd for extra early varieties, De Cicco and Green Mountain, 3.5 to 4 months for early varieties, Calabrese, Early K & V, Waltham 29, and K & V, and 4 to 4.5 months for medium varieties, Medium and Medium E. Plants sown in April needed a little more days for harvesting than those sown in May to Augtst. Seeds should be sown by early August for early varieties and by early July for medium varieties to get good yield around Tokyo.

Broccoli belongs to the cabbage group, which requires low temperature for flower bud formation. Early and medium varieties of broccoli, however, formed flower buds even in the warmest season of the year. It seemed that the low temperature requirements of those varieties were very little.

Late varieties, Medium Late and Late, formed curds during winter irrespective of the planting date. Those varieties seemed to require low temperature for flower bud formation.

12. Effect of gibberellin spraying on fruiting of vegetable fruits. Kazuhiko TAKAHASHI, Tadayoshi SUGIYAMA, Masao TADOKORO and Shunei RYO (Agr. & Hort. 34, 1579–1580, 1959)

With tomato, spraying of GB 5 and 10 ppm solution enhanced markedly the percentage of setting of both emasculated and non-emasculated flowers. The fruits thus obtained were, however, smaller in size than normal fruits. Spraying of PCA 1% solution increased fruit set, size and weight, and also hastened ripening, but the parthenocarpic fruits thus obtained were more puffy than normal fruits.

In the next step, spraying of the solution containing both GB and PCA (GB 10 ppm and PCA 1%) was tried. The result showed that the mixture solution increased fruit set, size and weight and hastened ripening as well as PCA alone did, and degree of puffiness was much lower than that of the fruits obtained by PCA spraying. It was suggested that spraying of mixture of GB and PCA was promising as the spray for improving fruit setting of tomato.

With cucumber and squash, no favorable effects were obtained by GB spraying.

13. Studies on the composts for raising seedlings of vegetable crops in hot beds. Part I. Rapid-made compost for raising tomato seedlings. Kazuhiko TAKAHASHI, Masao YOSHIDA

and Rikuro HIRAO (Journ. Hort. Assoc. Jap. 29, 27–36, 1960)

There has been no standard method in making compost for raising tomato seedlings in hot beds in Japan. The usual practice is to pile organic materials, fertilizers and soil in alternating layers during summer. To get a uniform mixture, the stack is turned orthogonally two or three times in autumn and winter. Because of this practice nutrients are leached by rainfall during this period of about 6 months, and much labor is required for piling and turning of the compost.

Experiments were conducted during 1955–1959 to find easier and more rapid ways of making compost. Tomato variety "Kurihara" was planted in the greenhouse in many types of composts made with various combinations of organic materials, soil conditioners, inorganic and organic fertilizers, and soils. The composts used in this study were made immediately before use.

1. Of the soils tested for making compost, volcanic ash soil was better than either clay loam (Arakida) or sand because of its superior physical properties. Leaf mold from broad leaved trees was a better organic material than either rice straw manure or peat moss. The best results were obtained from mixtures of organic material and soil in the ratio of 1 to 1 in volume. No effects were observed of sieving of organic materials or addition of soil conditioners or sand to the compost.

2. The use of soy bean, rape seed and fish oil cakes, and rice bran, commonly used as fertilizer in Japan, in the rapid-made compost is discouraging because the decomposition of these materials injured the seedlings. Also when calcium cyanamide was used serious injury to the seedlings occurred. This was probably due to the decomposition of calcium cyanamide to dicyandiamide. The best source of nitrogen is from ammonium sulphate or urea. Injury was produced at higher rates of nitrogen than 430–870g N per cubic Ken (about 6,000 *l*) in volcanic ash soil and leaf mold in 1 to 1 ratio. Good growth of seedling was obtained when 4,300g of P_2O_5 per cubic Ken from super phosphate or fused phosphate was added. However, larger quantities of phosphates produced no injury. Fertilization with K_2SO_4 and KCl at the rate of 430–870g K_2O per cubic Ken, gave excellent growth but higher rates produced poor seedlings.

3. Field experiments were carried out in 1958–1959 at the Tokyo University Farm at Tanashi. Tomato variety "Furuya-Wase" was sown in hot beds in the ordinary compost and in the rapid-made compost of volcanic ash soil and leaf mold (1 to 1 ratio) with optimum quantities of N, P_2O_5 and K_2O. These seedlings were transplanted to the field and grown to maturity. The early and total yields showed no significant difference in the two treatments indicating that the rapid-made compost was the same as the ordinary compost.

14. Studies on the classification of Japanese lawn grasses. Part 1. On the horticultural classification of Japanese lawn grasses. Fumio KITAMURA (Journ. Jap. Inst. Landscape Architects, 23 (1), 6–9, 1959)

Japanese lawn grasses have not yet been accurately classified. The author has studied their morphological and physiological characters from a classificatory point of view and found that the average value of the width of the leaf is the best standard of classification. Based on this the author divided them into four groups as follows:

(1) Large-leaved lawn grasses (Wild lawn grasses, No-shiba group)
......varieties of *Zoysia japonica* STEUD.
Leaf width....over 4.0mm.

(2) Medium-leaved lawn grasses (Park lawn grasses, Chū-shiba group)

......varieties of *Z. Matrella* MERR.
Leaf width....2.5–4.0mm.

(3) Small-leaved lawn grasses (Garden lawn grasses, Chōsen-shiba group)
......varieties of *Z. Matrella* MERR.
Leaf width....1.0–2.5mm.

(4) Slender-leaved lawn grasses (Potted lawn grasses, Hime-shiba group)
......varieties of *Z. tenuifolia* WILLD.
Leaf width....under 1.0mm.

15. Fundamental studies on the culture of American and European lawn grasses in Japan. Part 1. On the germination of the seeds of American and European lawn grasses and the repression effects on their growth. Fumio KITAMURA and Tomoo OZAWA (Journ. Jap. Inst. Landscape Architects, 23 (2), 11–15, 1959)

In these studies, the authors made some experiments on the germination of the seeds of American and European lawn grasses (Kentucky bluegrass, colonial bentgrasses-Astoria bent and Highland bent, redtop, Bermudagrass, Chewings fescue and Italian ryegrass) in Japan and on repression effects on their growth, and the following results were obtained.

(1) According to the size and the weight of the seeds, plants were arranged as follows:
Size: Italian ryegrass > Chewings fescue > Kentucky bluegrass > redtop > Bermudagrass > colonial bentgrasses.
Weight: Italian ryegrass > Chewings fescue > Kentucky bluegrass > Bermudagrass > redtop > colonial bentgrasses.

(2) In germination percentage, Chewings fescue, Italian ryegrass, redtop and Astoria bent were good, Highland bent and Bermudagrass were somewhat bad, and Kentucky bluegrass was the worst. As the seeds of Kentucky bluegrass and redtop became old, their germination percentages became low. Bermudagrass showed the highest percentage in autumn of the second year. Chewings fescue showed the highest percentage in spring of the third year, and so did Italian ryegrass. The result of the experiment shows that most of American and European lawn grasses have dormant period of some duration. Except a few species, the seeds of American and European lawn grasses are available after the preservation of 2 or 3 years.

(3) The result of repression experiments was as follows:
By the repression after sowing, the growth of American and European lawn grasses were quickened. Their weight (fresh and dry measure), the height of the plants, the amount of stems and stolons, and the length and the amount of roots increased. The best results were obtained in the following lawn grasses.

	Weight	Area	Duration
Highland bent	100 kg	2500 cm^2	1 minute
Kentucky bluegrass	150 kg	2500 cm^2	1 minute
Others	200 kg	2500 cm^2	1 minute

16. Effects of gibberellin on tne growth and the flowering of several evergreen trees and shrubs. Fumio KITAMURA (Journ. Jap. Inst. Landscape Architects, 23 (3), 5–8, 1960)

Experiments were carried out in order to investigate the effects of gibberellin on the growth, the flowering and the cold-resistance of several evergreen ornamental trees and shrubs.

Gibberellin was applied to the plants by spraying as aqueous solution once for all and monthly repeatedly (from August to October). Concentrations of solution of gibberellin were 50 and 100 ppm. Comparison was made between treated plants and non-treated control plants.

The results obtained are as follows:

(1) The growth of them was generally more increaced in high concentration or by the repeated application of gibberellin solution than that of non-treated plants. The effect of growth-increase was in order from "Otome-tsubaki (*Camellia japonica* L. var. *rosacea* MAKINO)", "Winter Daphne (*Daphne odora* THUNB.)", "Evergreen Spindle-tree (*Euonymus japonicus* THUNB.)" to "Cape Jasmine (*Gardenia jasminoides* ELLIS forma *grandiflora* MAKINO)".

(2) Flowering of Winter Daphne was remarkably hastened by spraying of gibberellin solution. The higher the concentration or the more repeatedly the application of gibberellin solution was, the more the flowering of Winter Daphne was hastened. The remarkably hastened flowering was about five months earlier than the normal flowering.

(3) Cold-resistance of Cape Jasmine was weakened by spraying of gibberellin solution. The higher the concentration or the later the spraying of gibberellin solution was, the more the cold-resistance of them was weakened. A few of them were dead.

17. Some characters of indigenous rice varieties in Japan. Takane MATSUO, Koichi HASEGAWA and Tetsuya YAMADA (Jap. Journ. Breed. 9, 93–96, 1959)

Indigenous varieties gathered from some isolated islands and mountainous parts of Japan, which are thought to be cultured from year to year since near 100 years ago or more, were cultivated and observed their morphological and ecological characters comparing with several improved varieties of Japanese paddy rice and some of upland and foreign rice.

The shape of grains of indigenous varieties was almost the same as improved varieties which are the typical Japonica. However, the former have longer culms and spikes than the latter. The length of culm, panicle and leaf, the weight of ear, the number of rachis branches and tillers of indigenous varieties show wide variations as foreign rice. The plant type of indigenous varieties, as the result, resembles to them.

Though the reaction of paddy to phenol and solubility of grains by KOH are used as the discriminant characters between the Japonica and Indica type of rice, some of indigenous varieties reveal positive to phenol and negative to KOH as some ones of foreign rice. The photoperiodic response and sexual affinity of the indigenous rice also were investigated on the phylogenic point of view. One of them has a large basic vegetative growth habit and low sensitivity for the short-day treatment. The sexual affinities of them to 2 Indica varieties also are varied in wide range.

The indigenous varieties of Japan seem to have many genotypes in morphological and ecological characters as foreign rice and breeders in Japan improved them to the special genotypes having the short culm, many spikes, awnless and short-narrow leaves.

18. **A report on the after-effects of growth on the next generation in rice plants (*Oryza sativa* L.) cultivated under the continuous low nutritional condition.** Takane MATSUO, Tetsuo NAKAJIMA and Yuzo FUTSUHARA (Jap. Journ. Breed. 9, 113–117, 1959)

Rice variety Norin 33 of the same origin was cultivated through its whole growth period under the nutritional condition of nitrogen or phosphoric acid deficiency for a few generations,

and then tested for their after-effects on the next generation grown under the condition of complete nutrition in regard to their growth and heading date. The results were summarized as follows.

(1) It was recognized that the deficiency in nitrogen or phosphoric acid was inhibitory for growth of rice plants, and the inhibitory effects of the continuous exposure to the deficiency in nitrogen or phosphoric acid were cumulative from generation to generation.

(2) The cumulative inhibitory effects were recognized in the next generation grown under the condition of the complete nutrition. The after-effects, however, faded away after the cultivation under the complete nutritional condition for one generation.

(3) In regard to heading date, the accidental earliness was recognized on the next generation of the plants grown under the nutritional condition of phosphoric acid deficiency for three generations. However, such earliness was not genetical, because the earliness was faded away after the cultivation under the complete nutritional condition for one generation.

19. **A comparison of effectiveness of selection in rice hybrids between pedigree, mass-selection and bulk system of breeding method.** Takane MATSUO, Tetsuo NAKAJIMA, Akira KOMATSUZAKI and Kiyoyasu SUGAWARA (Jap. Journ. Breed. 10, 33-41, 1960)

A hybrid population between a Japanese rice and a Chinese rice was treated by each of the 3 systems of breeding method; pedigree, mass-selection and bulk. Individual selection and bulk of seeds were repeated in each following generation untill the F_6. Population in bulk system were raised in 3 locations from F_2 to F_6 where the climatic and biological environmental conditions were different, and individual selection was done at each location in F_6. In F_7 and F_8 generations, the performance test was conducted for all the selected lines from each systems to compare the effectiveness of selection between these systems.

The results show that the pedigree system had more lines with high yield, strong culm or early maturity as compared with the bulk system. The inferiority of the bulk system in the effectiveness of selection may mainly due to : 1) the weakness of individuals with good characters in the competition of bulk population, 2) the shortage of number of individuals in F_6 generation and that of lines in F_7 used in bulk system and 3) the effectiveness of individual selection for the grain-weight paralleled with plant-type in F_2 of the pedigree system. By the mass-selection system, on the other hand, there were many heterozygous lines in F_7 though they were good in growth and yield.

The results also revealed that: 1) the parent-offspring correlation coefficients calculated in the pedigree system were large for heading-time, culm-length, spike-length, quality of brown rice and disease resistance and small for grain-weight and number of spikes, 2) the natural selection for the agronomical characters, especially for heading time and disease resistance, in bulk populations was different by the environmental conditions of locations where the hybrid populations were grown.

20. **A developmental analysis of yielding ability in varieties of field crops. Part 1. Leaf area per plant and leaf area ratio.** Shigesaburo TSUNODA (Jap. Journ. Breed. 9, 161-168, 1959)

An attempt has been made to account for the variation of yield in terms of growth and development of the crop plant, with special reference to the varietal response to manuring. Six varieties of rice, ten varieties of sweet potato and twelve varieties of soyabean were used as main materials.

Throughout these three crops, varieties adapted to light manuring tended to have a larger leaf area per plant at least during the earlier growth period than those adapted to

heavy manuring. In a varietal trial of sweet potato, it was observed that varieties having a high ability of leaf expansion could not necessarily yield high under heavily manured conditions, because (1) under such conditions increase in the leaf area did not necessarily result in an increase in total dry matter production and (2) the high ability of leaf expansion tended to be combined with a lower migration coefficient, i. e., a lower ratio of tuber yield to total dry matter production.

Furthermore, the results showed that the varietal difference in leaf area, at least among the prostrate varieties of sweet potato, was strongly affected by the internal factors that determine the varietal difference in leaf area plant weight ratio. Through interpreting the leaf area plant weight ratio in terms of its components, we may get a deeper knowledge on the relationship between the varietal difference in leaf area and the varietal yielding ability, including the problem of "migration coefficient".

Part 2. The assimilation-system of plant as affected by the form, direction and arrangement of single leaves. Shigesaburo TSUNODA (Jap. Journ. Breed. 9, 237–244, 1959)

As the aspect of an assimilation-system at the chloropast-cell-tissue level depends on (1) form, (2) direction and (3) arrangement of single chloroplasts, the aspect of an assimilation-system at the leaf-plant-plant population level seems to be expressible by means of the following three attributes of leaves, i. e., the form, direction and arrangement of leaves.

Further, it seems possible to formulate the following two types of assimilation-system at the leaf-plant-plant population level; one is the combination of "thicker and smaller" leaves, near "vertical" position of leaves and "gathering-type" arrangement of leaves, and the other is the combination of "thinner and larger" leaves, near "horizontal position of leaves and "dispersing-type" arrangement of leaves. The former is the assimilation-system of the typical varieties adapted to heavy manuring (e. g., Ginbozu-midashi, semi-dwarf variety of rice), and the latter is the assimilation-system of the typical varieties adapted to light manuring (e. g., Mao zu tao, rice variety belonging to the Indica type ; sweet potato varieties adapted to light manuring).

The former type is ideal when the mesophyll tissues can be produced abundantly per unit area of land as in the case of heavy manuring, being suitable to dispense the incident solar rays as equally as possible among all the leaves and among all the photosynthetic mesophylls of the plants. While, the latter type is ideal when the total amount of mesophyll tissues produced per unit area of land is small as in the case of light manuring, though increasing the amount of light available for the plants.

Part 3. The depth of green colour and the nitrogen content of leaves. Shigesaburo TSUNODA (Jap. Journ. Breed. 10, 107–111, 1960)

This paper deals with the depth of green colour of leaves in varieties of field crops in relation to their fertilizer response.

The lightness of the upper leaf surface was graded visually by using an achromatic value scale based on the Japan Engineering Standard, and it was observed in rice as well as in sweet potato that varieties adapted to light manuring tended to have light coloured leaves in comparison with those adapted to heavy manuring, though this tendency was not marked in the case of soyabean.

In the previous paper, it has already been pointed out that varieties adapted to light manuring tended to have "thin", "horizontal" and "thinly dispersed" leaves. All these situations of leaves tend to bring about a light saturation except in a shady place. It may

be attributable to the above fact, that varieties adapted to light manuring should not or need not be coloured so deep.

Besides, as in the cases of many other workers, a parallelism between the nitrogen content and the depth of green colour of leaves was observed in the varietal comparisons of rice and sweet potato in this study.

21. **The estimate of the segregation of seedling mutants in the second generation of irradiated rice.** Hikoyuki YAMAGUCHI (Jap. Journ. Breed. 9, 128–134, 1959)

In the field of "mutation breeding" in normally self-pollinating cereals, which takes aim at the utilization of the genetic variation increased with the application of ionizing radiation, some spikes have been picked up from the individual plant in X_1 (treated) generation and each spike has been sown separately as one strain for the detection of various deviants appeared in the X_2 generation.

It is expected that about 25% of the grains of a spike will be homozygous for mutated allele, from the description that the vast majority of induced mutants are completely or partially recessive to the original type. Therefore, NYBOM (1954) reported that the spikes of X_1 plants were sown separately in X_2, being one strain with maximum 20 plants from each of barley. Although the minimum number of plants necessary to find one mutant out of a certain mutated strain in X_2 is determined by the knowledge of the segregating proportion of homozygous plants for the mutated allele, there are some difficulties to estimate directly the above proportion for the decrease of kernels in X_1-spike due to radiation-induced sterility. Accordingly, the proportions of chlorophyll mutants in X_1-spike strain, which were produced from the irradiated seeds of rice, were estimated by using FISHER's sib method. This estimate gave 0.2305 ± 0.0084 for X irradiation and 0.1774 ± 0.0092 for the treatment of thermal neutrons of Rikuu No. 132 and also 0.1770 ± 0.0111 for the irradiation of thermal neutrons of Norin No. 29. Two proportions in the case of neutron-exposured seeds deviated very significantly from the value of 0.25 that would be expected if mutated character was a simple recessive. The deficiency of mutants is probably caused by gametic elimination after meiosis. It is interesting that the defective transmission for mutated character seems to demonstrate fundamental differences in the mode of action of two kinds of ionizing radiations; that is, thermal neutrons seem to induce in much greater relative abundance either the simultaneous mutations of two closely linked loci, one of which being the chlorophyll mutation and the other the deleterious mutation upon the development of gamete, or small deficiencies.

As a result of the calculation based on the above-given estimates, the number of X_2 plants in one spike-strain necessary to detect at least one mutant within a mutated strain with the probability of 99% was attained 18 plants in a case of X irradiation and was obtained 24 plants in a case of the exposure of thermal neutrons.

22. **Radio-sensitivity of gamma-irradiated autotetraploid in rice.** Hikoyuki YAMAGUCHI and Akihiko ANDO (Jap. Journ. Breed. 9, 169–172, 1959)

There are many reports on a comparison of the radiosensitivity among different ploids, and they are consistent in a conclusion that polyploids are more resistant to ionizing radiation than the diploids.

In this experiment, dry seeds of a diploid rice and its autotetraploid were treated with γ-rays from ^{60}Co, and radiosensitivity between diploid and tetraploid was compared by measuring various characters in the treated generation.

Consequently, diploid rice was more susceptible to γ radiation as compared with the tetraploid, and it is probable that a tetraploid cell has several radiation sensitive sites even if diploid has a few sites.

With due regard to no appearance of seedling mutation in the second generation of autotetraploid rice, the radiation resistance of tetraploid to γ ray could be ascribed only to the higher number of chromosomes.

23. **Genetic studies on *Cosmos bipinnatus*. III. Environmental modification on flower types in relation to temperature.** Yoshihiko SAMATA (Jap. Journ. Breed. 9, 179–186, 1959)

The influence of temperature upon the expression of genes in relation to flower-types has been investigated by using materials whose genotypes are known from gene analyses. Experiments were conducted in rooms where temperature was artificially kept at 30°C, 20°C, 10–13°C as well as in natural temperature (out of doors). Fifteen strains of different flower-types were propagated from cuttings, and the comparison of flower-heads was made within the same clone. Remarkable changes of flower-types was observed in malformed petaled types. Higher temperature brought about non-petaled type from vestigial-petaled or tubular-petaled type, while lower temperature produced highly developed tubular-petaled type. The most conspicuous changes were brought about in the semi-doubled type of flower-heads. A lower temperature produced flowers highly-doubled but with no pollen, while a higher temperature produced flowers of the apparently single type with abundant pollen. According to these results, it was postulated that what was affected by temperature might possibly be the character which is under the control of minor genes whose various expressions are subject to changes of temperature.

24. **On the difference of the expression of multi-lunar marking in several silkworm races.** Narumi YOSHITAKE (Jap. Journ. Genet. 34, 187–194, 1959)

In order to examine the dependence of the expression of a multi-lunar gene (*L*) on a variety of genetical backgrounds, crosses were made between each three multi-lunar strains ($H9L^{RO}$, H2L and H7L) and several normal strains of different origin.

The expression of the multi-lunar marking was the highest in crosses with Tropical and Cantonese races followed by crosses with Chinese, Japanese and European races, decreasing in the mentioned order down to the lowest in crosses with trimoltine races. Such a relationship became more obvious after repeated back-crossing with the same strains.

These results suggest that the expression of *L* gene is more influenced by several other genes than its normal allele, and that some of these genes may have some relation with voltinism.

It seems likely that the genetical background of such nature is more or less similar within strains belonging to the same local race, while it is not between races of different localities.

25. **Immunogenetic studies of races in the silkworm, *Bombyx mori*.** Narumi YOSHITAKE (Journ. Sericul. Sci. Jap. 28, 204–210, 1959)

A comparison of antigen components in various races using precipitation test was carried out in order to know the relationship among the races of the silkworm. As a result, 7 kinds (A-G) of antigen components were discriminated except for the common antigen (Table).

Japanese and Chinese races have A antigen in common, while Japanese and European races have B and C. It was found that Cantonese and Tropical races are closely related

with Chinese race. From the above-mentioned results, the races of the silkworm can roughly be divided as follows: Japanese-European race group, and Chinese-Tropical race group.

Strain \ Antigen	Common	A	B	C	D	E	F	G
Bagdad	+	−	+	+	−	−	−	+
N 1	+	+	+	+	−	−	−	−
Benyo	+	+	+	−	+	−	−	−
C 106	+	+	−	−	+	+	+	−
Daizō	+	−	−	−	+	+	−	−
Taiwan No. 8	+	−	−	−	+	−	+	−

26. **Induction of polyhedroses by temperature treatment in the silkworm, *Bombyx mori* L.** Toshihiko HUKUHARA and Hisao ARUGA (Journ. Sericul. Sci. Jap. 28, 235–241, 1959)

When fifth-instar larvae of the silkworm before first feeding after ecdysis are subjected to low temperature (0–3°C) for 16 or 24 hours, both nuclear and cytoplasmic (mid-gut) polyhedroses break out with much higher frequency than that in the control. Intermittent cold-treatment is more effective than continuous one in the induction of polyhedroses. Silkworm stages play an important part in the efficiency of induction by cold-treatment. Aptitude is low during moult, whereas after ecdysis it rapidly increases and then reaches a very high level which is maintained for certain period. Larvae fed with mulberry leaves after ecdysis show remarkably low aptitude. The results obtained by partial heat-treatment of larval body reveal that high temperature can induce the polyhedroses and that special organs or tissues situated in the anterior part of larval body play a part in the induction of polyhedroses. The process of induction could be visualized as follows. Temperature treatment disturbs the physiological condition of larvae, culminating in an abnormal condition which in turn causes the transition of the viruses from a non-infective to an infective state.

27. **Difference of induction rate of polyhedroses by low temperature treatment between inbred lines and their hybrids in the silkworm, *Bombyx mori* L.** Hisao ARUGA and Hitoshi WATANABE (Journ. Sericul. Sci. Jap. 28, 302–307, 1959)

Among various inbred strains of the silkworm, Daizō and Shungetsu are resistant to both nuclear and cytoplasmic (mid-gut) polyhedroses, while Ryō and Shūka are susceptible to both polyhedroses, especially to cytoplasmic one. Percentages of polyhedroses in the fifth instar larvae of these four inbred strains and their F_1 hybrids, as well as both F_2 and backcrossed hybrids, were measured. Each percentage of polyhedroses was converted into MATHER's potence for the sake of ready comparison of the resistance between hybrids and their parental lines.

The results obtained from the test, under the ordinary rearing conditions, indicated that frequency of polyhedroses in F_1 hybrids is much less than their parental lines and that F_1 hybrids show heterosis for the resistance to the two kinds of polyhedroses. The results also suggested that the amount of heterosis for the resistance to cytoplasmic polyhedrosis is liable to be larger than that for the resistance to nuclear polyhedrosis. The heterosis for the resistance was, generally speaking, also observable in F_1 hybrids.

On the contrary, when the fifth instar larvae, immediately after ecdysis, were exposed to low temperature (5°C) for 24 hours before first feeding, the frequency of nuclear polyhed-

rosis was higher in F_1 hybrids than in inbred lines. And nuclear polyhedrosis in hybrids of resistant strains, induced by low temperature treatment, tended to break out with much higher frequency than that in hybrids of susceptible inbred strains. Regarding the frequency of cytoplasmic polyhedrosis, the above-mentioned tendency observed between F_1 hybrids showed medium frequency of parental lines.

28. **Studies on the induction of polyhedroses by the low temperature treatment in the silkworm, *Bombyx mori*.** Hisao ARUGA and Narihiko ARAI (Journ. Sericul. Sci. Jap. 28, 362–368, 1959)

Studies on the induction of nuclear and cytoplasmic polyhedroses by the cold-treatment were carried out using the silkworm larvae at several developmental stages in the spring and the summer seasons. The results obtained are as follows :

(1) No induction phenomena were observed in the larvae in the 1st, 2nd and 3rd moulting stages and the larvae soon after ecdysis of the 2nd and 3rd instars by the cold-treatment (5°C, 24 hours), but induction was recognized in the larvae of the 4th moulting and the larvae soon after ecdysis of the 4th and 5th instars, showing the highest induction rate in the stage of the 5th instar soon after ecdysis.

(2) Cytoplasmic polyhedrosis was considerably induced by cold-treatment for 6 hours carried out on 5th-instar larvae soon after ecdysis. This polyhedral disease was induced at considerably high rate in several stages of the 4th and 5th instars except for the cold-treatment of the later stages of the 6th day of 5th instar. It is interesting that the induction of the cytoplasmic polyhedrosis was recognized not only in the stage of no feeding but also in several stages of feeding of mulberry leaves.

(3) Nuclear polyhedrosis was induced by the cold-treatment in the stages soon after ecdysis and of the 1st day and from the 5th to 6th day after ecdysis of the 5th instar.

(4) In the 5th instar, the induction experiments by cold-treatment were carried out to clarify the relationship between the length of starvation after ecdysis and the rate of induction. The rate of induction of both nuclear and cytoplasmic polyhedroses of the larvae treated by the low temperature for 0–6 hours after ecdysis was lower than that of the larvae treated for 6–48 hours after ecdysis. From the above-mentioned results, it is plausibly thought that the rate of induction by the cold-treatment may be influenced by the change of physiological function accompanying with the lapse of time after the 4th moult, and this physiological change might be controlled by some hormonal substances.

29. **On the relationship between physiological conditions of larvae and the induction of polyhedroses in the silkworm, *Bombyx mori*.** Hisao ARUGA, Eiichi KANAI and Amnuay ISRANGKUL NA AYUDHYA (Journ. Sericul. Sci. Jap. 28, 369–374, 1959)

It may be considered that genetical, physiological and environmental factors are closely related to the appearance of both nuclear and cytoplasmic polyhedroses in the silkworm. Cold-treatment for 24 hours to the fifth instar larvae before first feeding after ecdysis induces those polyhedroses with high rate.

It has been ascertained that the rate of the appearance of both polyhedroses in natural condition was changed by the difference of some nutritional factors having relation with the quality of mulberry leaves and the same phenomenon was observed in the experiments of the artificial induction carried out by changing nutritional factors before and after cold-treatment.

As to the relationship between the nutritional conditions of larvae before refrigeration and the induction rate of polyhedroses, the results showed that when the nutritional factors

of larvae before refrigeration were considered to be relatively good, the appearance of nuclear polyhedrosis was high, and that of cytoplasmic one was low, but if the nutritional factors were relatively bad, the tendency of the induction polyhedroses showed the reversed relation. On the relation between the nutritional conditions of larvae after refrigeration and the induction of nuclear polyhedrosis good conditions of the factors showed the higher induction rate than the bad ones.

From the above-mentioned results it might be plausibly thought that the physiological conditions having relation with the nutrition of the silkworm larva play an important role in the spontaneous appearance, and the induction by the cold-treatment of polyhedroses.

30. Induction of nuclear and cytoplasmic polyhedroses by feeding of some chemicals in the silkworm, *Bombyx mori* L. Hisao ARUGA and Toshihiko HUKUHARA (Journ. Sericul. Sci. Jap. 29, 44–49, 1960)

Eighteen chemicals were tested for inducing effect of nuclear and cytoplasmic polyhedroses by feeding them to fifth-instar larvae of the silkworm, *Bombyx mori* L. Among them six were found to be effective in the induction of cytoplasmic polyhedrosis. They were sodium cyanide, sodium fluoride, arsenic acid, monojodoacetic acid, sodium azide, ethylene diamine tetraacetic acid (EDTA) and its disodium salt. The last two chemicals induced cytoplasmic polyhedrosis with high frequency when they were fed to fifth-instar larvae but only with low frequency when fed to fourth-instar larvae. In the induction by these two chemicals the larvae reared in Tokyo developed only cytoplasmic polyhedrosis, however those reared in Ueda developed both nuclear and cytoplasmic polyhedroses. When cold-treatment was carried out on fourth-instar larvae that had been fed with disodium salt of EDTA, sodium cyanide or mercuric chloride during the third instar, they developed cytoplasmic polyhedrosis with higher frequency than the control larvae.

31. Temperature effects on the heterosis of starvation resistance in the silkworm, *Bombyx mori*. Hitoshi WATANABE (Joun. Sericul. Sci. Jap. 29, 58–62, 1960)

The experiments were carried out to clarify the temperature effects on the heterosis in the starvation resistance, using several instar larvae immediately after ecdysis in some F_1 hybrids and their parental inbred lines being starved at 30°C, 20°C and 13°C respectively. The starvation resistance was compared by LT-50 of starvation-mortality.

F_1 hybrids showed overdominance for starvation resistance in each instar and at three kinds of temperatures, except the second instar larvae starved at 30°C. The amount of heterosis in early larval stages were larger at 13°C than at 30°C, and also the amount of heterosis in newly hatched larvae was affected by maternal cytoplasm. On the contrary, in late larval stages the amount of heterosis being free from maternal cytoplasmic effect was less at 13°C than at 30°C.

32. Difference in variability of some characters between inbred lines and their hybrids in the silkworm, *Bombyx mori*. Hitoshi WATANABE (Joun. Sericul. Sci. Jap. 29, 113–119, 1960)

Investigations were made to clarify the difference in variabilty of body weight, cocoon weight and cocoon-layer weight between F_1 hybrids and their parental lines. The results were summarized as follows:

Regarding the male or female groups, in weights of the 5th instar larva soon after ecdysis, pupa, cocoon and cocoon-layer, F_1 hybrids were less variable than parental lines. As for the group mingled with male and female, the tendency mentioned above was likely observed in weights of the 4th and 5th instar larvae soon after ecdysis and cocoon-layer

though it was not so obvious in weights of pupa and cocoon. In this case, for the reason why variabilities in weights of pupa and cocoon of F_1 hybrids were not significantly different from those of parental lines, it might be thought that the sex differences in those characters were larger and the frequencies in F_1 hybrids were more distinctly bimodal than those in parental lines so that each F_1's variation was enable to separate easily into two components, male and female groups.

There was no correlation between the amount of heterosis and variabilities in body weight, cocoon weight and cocoon-layer weight. But from the above-mentioned results, it might be thought that F_1 hybrids showed higher developmental homeostasis than their parental lines.

33. **Studies on nuclear polyhedroses and their inductions in some Lepidoptera.** Hisao ARUGA, Narumi YOSHITAKE, Hitoshi WATANABE and Toshihiko HUKUHĀRA (Jap. Journ. Appl. Ent. Zool. 4, 51–56, 1960)

Investigations were carried out to find out polyhedroses in some lepidopterous larvae. Nuclear polyhedroses were observed in *Barathra brassicae*, *Plusia nigrisigan*, *Hyphantria cunea* and *Antheraea pernyi*, but no cytoplasmic polyhedrosis could be detected.

No cross-infection of polyhedrosis was observed among *Barathra brassicae*, *Hyphantria cunea* and *Bombyx mori*, although each species could be infected when fed with the polyhedral bodies of the same species.

When the larvae of *Barathra brassicae* and *Hyphantria cunea* were exposed to low temperature (5°C) for 1–2 days, no polyhedrosis was induced. But nuclear polyhedrosis was induced in *Hyphantria cunea* by feeding chemicals such as arsenic acid, merculic chloride, Phygon, EDTA (ethylene diamine tetraacetic acid) and sodium salt of EDTA.

34. **Electrical properties of the cockroach giant axon.** Teruo YAMASAKI and Toshio NARAHASHI (Journ. Ins. Physiol. Lond. 3, 230–242, 1959)

Whereas the electrical properties of nerve and muscle fibres have received much attention, only a few data are available for those of insect nerves. In order to elucidate the mechanism involved in the excitation of the cockroach giant axon, its electrical properties have been examined by means of intracellular microelectrodes. One and the same microelectrode was used for stimulating and recording by employing a Wheatstone bridge circuit.

A delayed rectification is observed when the intensity of cathodal current exceeds a certain level. Furthermore a local potential, which is followed by an after-positivity, is set up by a subthreshold cathodal current. The membrane resistance is increased by raising the intensity of anodal current. With a very strong anodal current, the electrotonic potential decreases after reaching a maximum showing a hump.

The mean critical depolarization is 22 mV, and the mean threshold current is 4.4×10^{-8} A.

The electrical constants are as follows: specific membrane resistance 800 $\Omega\,cm^2$, specific membrane capacity 6.3 $\mu F\,cm^{-2}$, time constant 4.2 msec and space constant 0.86 mm.

The magnitude of the action potential is increased by hyperpolarization and decreased by depolarization.

The maximum rate of rise of the action potential undergoes little change by hyperpolarization, but is decreased by depolarization.

The after-positivity is increased by depolarization and decreased by hyperpolarization. The after-negativity is decreased by depolarization and increased by hyperpolarization. A straight line relating the after-positivity to the membrane potential has a slope of about unity under hyperpolarization, whereas a straight line for after-negativity has a slope of

less than unity hyperpolarization.

The calculated values of 6.2 x 10^{-12} M cm^{-2} $impulse^{-1}$ and 6.9 mA cm^{-2} are obtained for the total quantity of sodium entering the axon during an impulse and for the net ionic inward current during the rising phase of an action potential, respectively.

The nature of the delayed rectification, the mechanisms of the after-positivity and the after-negativity productions, and the peculiarities of the electrical properties in the roach giant axon are discussed.

35. **Nervous and cholinesterase activities in the cockroach as affected by demeton and methyldemeton.** Toshio NARAHASHI and Teruo YAMASAKI (Jap. Journ. Appl. Ent. Zool. 4, 64–69, 1960)

A number of investigations have so far been made on the inhibition of cholinesterase (ChE) by organophosphorus insecticides. There is, however, some doubt whether the inhibition of ChE is responsible for changes in nervous activity. This problem forms the basis of the present study. The effects of thiono- and thiol-isomers of both demeton and methyldemeton on the activity of the nerve cord of the American cockroach and on the ChE activity were studied.

Both isomers of methyldemeton cause an increase in spontaneous activity and a prolongation of synaptic after-discharge, which are followed by a burst of discharges and a final synaptic block. These effects are reversible on washing with normal Ringer's solution when a weak concentration is used.

Both isomers of demeton exert almost the same effect as those of methyldemeton, but differ in that the prolongation of synaptic after-discharge is preceded by a brief period of depression of post-synaptic response.

Four isomers all inhibit ChE of the nerve cord *in vitro*, concentration for 50 per cent inhibition being of the order of 10^{-6} M.

The ChE activity of nerve is half inactivated at the moment when spontaneous activity is increased and synaptic after-discharge is prolonged after treatment with demeton or methyldemeton.

It is concluded that the inhibition of ChE is mainly responsible for the changes in nervous activity. Additional mechanisms are suggested for the action of demeton.

36. **Synaptic transmission in the last abdominal ganglion of the cockroach.** Teruo YAMASAKI and Toshio NARAHASHI (Journ. Ins. Physiol. Lond. 4, 1–13, 1960)

In the present article an attempt has been made to analyse the electrical phenomena associated with the synaptic transmission and to clarify the nature of synaptic transmission in the last abdominal ganglion of the cockroach. Both local and propagated responses were recorded from the ganglion before and after treatment with various drugs, and the cholinesterase (ChE) activity of the nerve cord was determined.

A presynaptic volley sets up excitatory postsynaptic potentials (e. p. s. p. 's). When the e. p. s. p. reaches a certain critical level action potentials of the giant axons are generated. The mean value of synaptic delay is 1.59 msec. The delayed e. p. s. p. 's superimposed with discharges of small axons are observed with a latency of about 30 to 50 msec.

Phenobarbital and urethane are effective in prohibiting the discharge from ganglion cells keeping the pure e. p. s. p. to be observable.

At the onset of weak repetitive stimulation there is a brief phase of potentiation during which impulses are discharged. A depression follows and persists for a long time.

An application of anticholinesterases such as eserine, tetraethylpyrophosphate and parathion causes a marked increase and prolongation of the e. p. s. p. superimposed with a prolonged after-discharge. The e. p. s. p. grows so markedly that a persistent ganglionic depolarization associated with synaptic block is eventually brought about. A repolarization associated with recovery of the synaptic transmission occurs after some time.

Inactivation of ChE in the nerve cord after treatment with anticholinesterases runs parallel with the synaptic after-discharge.

When applied to the intact ganglion, the threshold concentration of acetylcholine for discharging impulses is as high as 10^{-2}. However, when the desheathed ganglion is eserinized, it is lowered to 10^{-4}.

The nature of transmitter substance in the last abdominal ganglion is discussed, and three possible explanations are suggested ; the possibility of acetylcholine as a transmitter still remains.

37. **Mechanism of the after-potential production in the giant axons of the cockroach.** Toshio NARAHASHI and Teruo YAMASAKI (Journ. Physiol. Lond. 151, 75–88, 1960)

The action potential of the cockroach giant axons has been analysed with special reference to the mechanism underlying the production of the negative after-potential.

The mean values for the membrane potentials are as follows : resting potential 77 mV, action potential 99 mV, positive phase 4.6 mV, negative after-potential 1.5 mV.

These four values are decreased by raising the external concentration of potassium.

During a train of impulses the spike height and the positive phase decrease nearly exponentially, and the negative after-potentials add in a linear manner.

The decay of a negative after-potential can be described by a single exponential term with a mean time constant of 9.2 msec. The time constant is decreased by raising the external potassium. The decay following a train of impulses shows in addition a terminal slow phase with a time constant as long as 500 msec or more.

Raising the external concentration of calcium causes a slight increase in resting and action potentials, a marked increase in negative after-potential, and a slowing of the maximum rate of rise of the action potential, whereas it has little effect on the maximum rate of fall.

In high-calcium solution the decay of the negative after-potential can be expressed as a single exponential term with a time constant slightly longer than that in normal Ringer's solution.

The negative after-potential can be interpreted as being due to the accumulation of the released potassium in the immediate vicinity of the nerve membrane. The increase in potassium concentration is estimated as about 2.0 mM.

38. **Mechanism of increase in negative after-potential by dicophanum (DDT) in the giant axons of the cockroach.** Toshio NARAHASHI and Teruo YAMASAKI (Journ. Physiol. Lond. 152, 122–140, 1960)

Although it has already been pointed out that DDT causes the negative after-potential to increase, little is known about the mechanism involved. The present paper gives the results of experiments whose aim was to elucidate this mechanism.

After treating a cockroach giant axon with DDT, the positive phase following the spike decreases and finally disappears, and the negative after-potential increases until it attains a maximum, although the spike phase undergoes little change. There is a brief period during which a repetitive response can be induced by a single shock. Thereafter, however, the rate

of fall of the spike potential slows considerably, and the negative after-potential finally begins to decrease. Conduction block is never observed, and the resting potential undergoes little change throughout.

Immediately after the repetitive period, damped oscillations are often seen on the declining phase of the increased negative after-potential.

In normal axons the time course of the decay of the negative after-potential following a single impulse can be expressed as a single exponential term. After treatment with DDT, however, the decay is initially slower than normal, and terminates in a faster phase.

After a train of impulses in a DDT-poisoned axon, the declining phase terminates in a very slow component which is less marked than in a normal axon, and there is little or no sign of the terminal fast phase seen after a single impulse.

Unlike normal axons, the negative after-potentials in the DDT-poisoned axon do not add linearly during a train of impulses.

Delayed rectification, which is always observed in the normal axons when cathodal current is applied, is found to be further delayed and partly suppressed in the DDT-poisoned axons. On cessation of cathodal current the electrotonic potential decays more slowly in the DDT-poisoned axons than in the normal ones.

A rise in external potassium concentration reduces the DDT-induced negative after-potential. In potassium-free solutions it is greatly augumented and a plateau appears.

A rise in external calcium concentration causes a further increase in the DDT-induced negative after-potential.

It is concluded that the increase in negative after-potential caused by DDT is not caused by a rise in potassium accumulation near the nerve membrane, but by a delay and suppression of the rise in membrane potassium conductance which accelerates the falling phase of the action potential.

39. **Die Moosmilben (Oribatei) aus Südjapan.** Jun-ichi AOKI (Bull. Biogeogr. Soc. Jap. 21(1), 1–22, 1959)

In order to examine oribatid mites the author received nine samples of *humus* from southern part of Japan, i. e, Ehime, Kagawa, Saga, Nagasaki and Miyazaki Prefectures. As a result of extraction by Berlese apparatus 20 species of oribatid mites were found. Among them the author recognized 8 species new for Japan and 11 species new for science.

40. **Record of oribatid mites from Japan.** Jun-ichi AOKI (Jap. Journ. Sanit. Zool. **10**, 127–135, 1959)

Up to date about 12 species of oribatid mites were recorded from Japan. In addition to them the author newly recorded 13 species from our country. They are *Camisia segnis*, *C. lapponica*, *Heminothrus targioni*, *Hypochthonius rufulus*, *Hypochthoniella paridulla*, *Trhypochthonius tectorum*, *Nanhermannia nana*, *Xenillus tegeocranus*, *Cepheus cepheiformis*, *C. latus*, *Oppia quadricarinata* and *Protoribates monodactylus*. For the convenience the author proposed the Japanese common names for each family of oribatid mites. The Japanese morphological terms were also standardized with a schema.

41. **Zur Kenntnis der Oribatiden im Pilz. I. Berichte über einige Arten aus Nikko.** Jun-ichi AOKI (Annot. Zool. Japon 32, 155–161, 1959)

The oribatid mites living mushrooms were examined at Nikko, in Tochigi Prefecture and 8 species of mites were found on 5 species of mushroom. *Cepheus cepheiformis*, *Belba* sp., *Scheloribates latipes*, *Chamobates pusillus* and *Achipteria distincta* n. sp. inhabited

only on genus *Coriolus*. 79% *Carabodes bellus* n. sp. collected was found on *Trametes*. 19% on *Polystictus*, and 2% on *Coriolus*.

42. **Eine dreikrallige Gattung der Familie Perlohmanniidae (Acarina : Oribatei).** Jun-ichi AOKI (Jap. Journ. Zool. 12, 507–511, 1960)

The family Perlohmanniidae is represented by one genus *Perlohmannia* and all known species of the world have one claw on each leg. It is therefore interesting that perlohmanniid mite with three claws has been discovered. I offer for this animal a new genus *Apolohmannia* and the new species was named as *A. gigantea*.

43. **Injury to plants caused by oribatid mites.** Jun-ichi AOKI (Jap. Journ. Appl. Ent. Zool. 4, 136–137, 1960)

We have few knowledge about oribatid mites living on plants. The author obtained them on various plants in Yamanashi and Tokyo. *Camisia segnis* (HERM.), *Neoliodes* sp., *Trichoribates* sp. and *Oripoda* sp. seem strictly arboricolous species. They were obtained by beeting method from Japanese cypress, Japanese ceder, camellia, yamabuki, bamboo-grass etc. *Trhypochthonius tectorum* (BERL.), *Cepheus cepheiformis* (NIC.), *Ceratoppia bipilis* (HERM.), *Pelops* sp. and *Galumna* sp. were found both in soils and on living trees. Are they injurious to plants or not? In this respect we have little information, but many individuals of *Camisia segnis* (HERM.) were found on tea plants at Asakawa in Tokyo and juice of tea leaves was observed in their digestive organ.

II. Agricultural Chemistry

Abstract Nos. 44—91

44. **On the colored material of fulvic acid.** Kenzo KOBO and Ryo TATSUKAWA (Zeitschr. f. Pflanzenernähr. Düng. Bodenk. 84, 137–147, 1959)

The chemical properties of the colored material obtained by extracting fulvic acid with butanol were studied.

1. The colored material is unstable in alkaline solution and does not show distinct skeletal structure in comparison with the humic acid.
2. From the results of elementary analysis, ultra-violet and visible ray absorption spectra, infra-red absorption spectra and other analyses, it was proved that the chemical constitution of the colored material of fulvic acid is substantially the same, irrespective of the difference in the humification degree of the corresponding humic acids.
3. It is assumed that the colored material has the possibility of transformation to the humic acid through the process of condensation and polymerization.

45. **A new method for determining free iron in paddy soils.** Teruo ASAMI and Kyoichi KUMADA (Soil and Plant Food, 5, 141–146, 1960)

In the determination of free iron in paddy soils using sodium hydrosulfite, the conventional method was found inadequate because of the possible formation of ferrous sulfide precipitates during the extraction. Several conditions were examined and following method using EDTA solution was proposed.

Take 0.5–2.0 g of air-dry soil into a 200 ml Erlenmyer flask. Add 3 g of sodium hydro-

sulfite and 100 ml of 0.02 M EDTA (disodium ethylendiamine tetraacetate). Place it on a boiling water bath and maintain at 70°C for 15 min with occasional shaking. After the treatment, filter the supernatant solution into a 250 ml volumetric flask, then transfer the residue on the filter paper and wash it 3 times with 1% sodium chloride solution. Fill up and mix well. Pipette an aliquot of the filtrate into a 25 ml volumetric flask, add 1 ml of 5% hydroxylamine hydrochloride, 2 ml of 0.1% *o*-phenanthroline and 1.5 ml of acetate buffer. Fill up and mix well. After 30 min (more time is necessary when much filtrate is pipetted), read the absorbancy at wavelength of 508 mμ and compare with the standard curve.

In this method, it is unnecessary to destroy EDTA or sodium hydrosulfite in the sample solution for colorimetric determination of iron. Comparison of this method with some others will be reported in the next paper.

46. Comparison of several methods for determining free iron in paddy soils. Teruo Asami and Kyoichi Kumada (Soil and Plant Food, 5, 179–183, 1960)

1) The method of determining free iron in paddy soils using sodium hydrosulfite and disodium ethylendiamine tetraacetate, established in the previous paper, was compared with other four methods. And it was explained that this method was desirable in the following respects.

a) This method gives the same amount of free iron irrespective of the amount of soil sampled, at least within the range between 0.5–2.0 g.

b) In general, the docomposition of soil organic matter is not necessary.

c) Repeated treatment is not necessary.

d) Procedure is very easy and rapid.

e) The data obtained by this method explain well the behaviour of free iron in paddy soils.

2) Inhibition of EDTA, oxalate mixture and sodium citrate to the iron determination using *o*-phenanthroline was examined, and it was concluded that in the 3 methods studied in this paper the decomposition of these chelating agents was unnecessary for determining iron in the extracted solutions.

47. Dynamic studies on the nutrients uptake by crop plants. Part 22. The growth of rice plant on poorly drained soil as affected by the accumulation of volatile organic acids. I. Shingo Mitsui, Kikuo Kumazawa and Noboru Mukai (Journ. Sci. Soil Manure, Jap. 30, 345–348, 1959)

On the basis of the previous studies and observations several volatile organic acids can possibly evolve in a sufficient amount to damage rice root, if the soil is of extremely poor drainage.

In this study, the conditions promoting the evolution of organic acids were investigated. They were: With versus without organic matter, sulfate versus non-sulfate fertilizers, cooled versus not cooled, and occasionally drained versus not drained.

The most remarkable result was that unexpectedly higher accumulation of volatile organic acids was detected in the low soil temperature condition compared with high, as the climatic temperature in general gradually increased.

The accumulation can be reasonably attributed to the lag of decomposition as compared with the production of soil organic acids, particularly in the low soil temperature.

The number of ears increased in the low soil temperature whereas the weight of straw decreased. The weight of ears decreased in both low temperature and sulfate addition.

In conclusion, the " AKIOCHI " like damage of rice plant grown on the soil of extremely

poor drainage can partially be explained by the accumulation of harmful organic acids under prevailing condition.

Part 23. The growth of rice plant with the use of poorly drained soil as affected by the accumulation of volatile organic acids. II. Shingo MITSUI, Kikuo KUMAZAWA and Takashi HISHIDA (Journ. Sci. Soil Manure, Jap. 30, 411–413, 1959)

The evolution in the soil of various volatile organic acids was investigated at low versus high temperature with the addition of a quantity of peanut seed cake in the laboratory.

The organic acids were fractionated by the help of silica gel column chromatography and determined.

The most remarkable result was that more organic acids such as formic, acetic, propionic and butyric acids were accumulated at low soil temperature as compared with higher.

A part of the harmful effect of the soil of extremely poor drainage can be attributed to this mechanism.

Part 24. Method of the determination of cation exchange capacity and its intensity of plant roots. Shingo MITSUI and Minoru UEDA (Journ. Sci. Soil Manure, Jap. 30, 487–491, 1960)

The results obtained are as follows:

1) The electrodialytic or hydrochloric acid pretreatment of the living plant roots showed almost the same values of C.E.C. and similar effect on the respiration activity of plant roots.

2) According to the titration curves it seemed most probable that the stronger exchange sites were distributed on the surface of plant roots, while the weaker exchange sites existed rather within the plant roots.

3) The C.E.C. of plant roots was considerably affected by the nutritional status and the growth stage.

4) The energy combining cations and the sites of plant roots depend on the combination and the ratio of coexisting cations.

Part 25. The effect of cation exchange capacity of soil and plant roots on the uptake of some cations, particularly of magnesium. Shingo MITSUI and Minoru UEDA (Journ. Sci. Soil Manure, Jap. 30, 525–530, 1960)

To know the effect of cation exchange capacity of plant root on the actual nutrient uptake, red bean with large C.E.C. and wheat with small C.E.C. were respectively sown on the magnesium defficient soils with two different C.E.C. The results obtained are as follows:

1) High correlation coefficient was found between the magnesium or calcium uptake and the C.E.C. of plant roots.

2) The uptake of calcium and magnesium was remarkably affected by wheat root, the C.E.C. of which was very small.

3) According to C.E.C. of plant roots the cation uptake differed in relation to the base status of the soils.

4) The uptake of magnesium, which is low in the soil, was promoted by elevating the saturation degree of C.E.C.

5) In case of wheat the uptake of calcium and magnesium was remarkably inhibited by the addition of potassium.

The above-mentioned results can clearly be explained on the basis of the cation exchange capacity and intensity of both soils and plant roots.

Part 26. The effects of light on the nutrients uptake by wheat seedlings, particularly in relation to the preliminary treatments. Shingo MITSUI, Kikuo KUMAZAWA, Shohei AOKI and Hiroshi HIRATA (Journ. Sci. Soil Manure, Jap. 31, 33–38, 1960)

The study consists of two experiments: the one is the simple effect of light on the nutrients uptake by wheat seedlings, and the other the same as influenced by preliminary illumination and nutrition.

In general, the effects of light at the time of absorption may be devided into three groups as follows.

NH_4-N, S, and H_2O increased almost proportionally to light intensity (group I); Ca, Mg being almost indifferent to light intensity (group II), and P, K showed unique responses (group III).

The pretreatment, however, modified the effect, especially for N, P, K.

On the whole, the low salt-plants absorbed more N, P, K, showing little relation to light intensity. But high salt high sugar-plants which were prepared by means of preillumination, accumulated K, N nutrients as much as low salt-plants or rather more at high light intensity.

O_2 uptake by roots increased in proportion to light intensity and this tendency was clearer in high sugar-plants than in low sugar-plants.

Conclusively, the effects of light are never the same for respective nutrient uptake. The light may increase transpiration and carbohydrates accumulation in plants which offer skeletons to synthesize proteins and other N-compounds, resulting in the increase of H_2O and N uptake in proportion to light intensity. On the other hand, P and K uptake depend on other factors which are not directly, but indirectly connected with photosynthesis, i.e. with some metabolic processes of its products depending upon the pretreatments.

48. The uptake and utilization of carbon by plant roots from ^{14}C-labelled urea. Part 2. The comparison of the uptake of carbon from urea and carbonate by plant roots. Shingo MITSUI and Kiyoshi KURIHARA (Journ. Sci. Soil Manure, Jap. 30, 405–410, 1959)

In order to investigate the uptake of carbon in urea through plant roots, the ^{14}C uptake supplied by labelled urea and bicarbonate in comparison with lowland and upland rice by semi-soil culture was carried out. Also, water culture with rice plant was conducted to avoid soil complication.

In the first experiment the rate of ^{14}C uptake was 4.47–13.2% for lowland rice under waterlogged soil condition as compared with 0.05–0.33% for upland rice under upland soil condition. This trend agrees well to the result obtained in previous reports. Moreover, the ^{14}C absorption from urea was almost doubled that from bicarbonate for respective soil condition.

As the lowland and upland rice belong to the same species, the principal difference can be reasonably attributed to the different soil conditions in relation to the loss of carbon dioxide. The favourable availability of urea ^{14}C as contrasted with bicarbonate ^{14}C was discussed on the basis of various factors considered.

In the second experiment the ^{14}C uptake during 24 hours amounted to 8.46% for bicarbonate and 3.59% for urea contrary to the first experiment. Autoradiograms and specific activities of translocated ^{14}C in the various parts of rice shoots indicated that its distribution was not significantly different between urea and bicarbonate, but the increased accumulation of ^{14}C was remarkable in the roots when supplied with urea. Further, 74% of supplied urea remaining in culture solution after experimentation and remaining NH_3-N was very little.

The second experiment most probably indicated that urea can be absorbed as such if the decomposition of urea into ammonium bicarbonate is not so rapid as in soil. The lower absorption rate of urea can be reasonably attributed to the urease activity within the root as revealed in the remarkable accumulation of ^{14}C in the root.

Part 3. Analytical experiments on differences in carbon uptake between upland and waterlogged soil conditions, and between urea and bicarbonate carbon. Shingo MITSUI and Kiyoshi KURIHARA (Journ. Sci. Soil Manure, Jap. 30, 427–432, 1959)

To analyse the differences in carbon uptake obtained in previous papers, the effect of pH values and stagnant water on CO_2 absorption from soil or culture solution by rice plant, and CO_2 escape into the atomosphere from soil without standing crop was investigated.

1. The uptake of ^{14}C from soil increased from pH 5.3 to pH 5.7, decreased between pH 5.7 and pH 6.7 and followed by the subsequent reincrease at further alkaline pH.
2. The uptake of ^{14}C from culture solution remarkably increased as pH decreased. However, a slight increase was also shown at alkaline pH.
3. According to the elevation of soil pH, the amount of supplied ^{14}C escaping into the air was depressed linearly, but the total amount of CO_2 escape did not always show such a trend.
4. Considerable difference of CO_2 escaping was observed either between waterlogged and upland soil conditions or between urea and bicarbonate. The amount of escaping $^{14}CO_2$ from waterlogged soil was higher than from upland soil, and the ^{14}C derived from urea was held in soil much more than the ^{14}C applied as bicarbonate under both conditions.

These experimental results indicate that not only the pH and soil conditions strikingly influenced upon carbon uptake by plant roots, but also they were good enough to explain the results obtained in previous paper.

49. The uptake of strontium-90 and calcium by lowland and upland rice from soil, and their distribution in the plants. Kiyoshi TENSHO, Ko-Ling YEH and Shingo MITSUI (Soil and Plant Food, 5-1, 1–9, 1959)

The pot experiments, using Sr-90 and Tanashi volcanic ash soil, of lowland and upland rice were conducted by three different ways of applying Sr-90; basic mixed application with the upper two-thirds of the soil, basic surface application and top dressing at the young panicle formation stage (for lowland rice only). In these experiments, we studied the absorption of Sr-90 and calcium through the roots and their movement and distribution in the plants, with major emphasis on comparison between these two elements.

1) The content of Ca and Sr-90 based on air dry basis is high in the stems and leaves, and extremely low in heads, particularly those of hulled rice. Both elements show uniform distribution in all parts of plants. That is to say, generally, the content of these two elements is the highest in lower leaf-blades, followed by upper leaf-blades, stem and sheath, husk, rachis, hulled rice, and polished rice in the order mentioned.

2) The ratio of Sr-90 to Ca, however, is not uniform in all parts of plants. It was shown that this ratio is lower in the seed part, that is, husk and hulled rice, than in any other parts, irrespective of the kind of plants and the method of Sr-90 application. No distinct difference was noticed in this ratio among bran, hulled rice and polished rice. Considering that this ratio is unusually low in the lower leaves of lowland rice in the top dressing pot, we made it clear that redistribution of Sr in plants is difficult.

3) The ratio of Sr-90 to Ca is slightly higher in plants for mixed application than in the exchangeable part of the upper two-thirds part of soil after harvesting, but the ratio

is lower in plants than in the hot-HCl extractable part of the soil.

4) The amount of Sr-90 absorbed into plants differs depending upon the method of applying Sr-90. In the case of lowland rice, this amount is the largest in top dressing followed by surface and then mixed application. In the case of upland rice, on the other hand, Sr-90 absorption is remarkably low in the surface application. Sr-90 absorption rate was 0.82–1.66% for lowland rice and 0.19–0.53% for upland rice.

5) The ratio of Sr-90 to Ca, depending upon the method of applying Sr-90, also showed a trend similar to the aforesaid trend in Sr-90 absorption. In the mixed application, however, no striking difference was noticed between lowland and upland rice.

6) From these results, we have indirectly verified that the unusually high S.U. value of contamination of the grains of rice and wheat, which was shown in the survey of Sr-90 contamination of crops, is not mainly through the roots, but is attributable to direct contamination caused by radioactive fallout. Moreover, it is suggestive that the superficial development of active root system of lowland rice may contribute to the higher contamination of Sr-90 via roots as compared with upland crops.

50. **The uptake of cesium-134 and potassium by lowland and upland rice from soil, and their distribution in the plant.** Kiyoshi TENSHO, Ko-Ling YEH and Shingo MITSUI (Journ. Sci. Soil Manure, Jap. 30, 253–258, 1959)

Pot experiments using ^{134}Cs of lowland and upland rice grown on Tanashi volcanic ash soil were carried out with special reference to the comparison between ^{134}Cs and K. ^{134}Cs was applied to the soil in two different ways, i.e., basic dressing mixed with the whole soil and basic surface application for upland rice, and in three different ways in lowland rice with an addition of top dressing on the standing water at the stage of young panicle formation.

Though the distribution of ^{134}Cs in the plants was similar to that of K, some different behaviours were clearly shown between two elements. On the air dry basis, the potassium content of stem and sheath was significantly higher than the other parts, but in the case of ^{134}Cs the almost uniform distribution was observed in the plants except brown rice.

$^{134}Cs/K$ ratios in various parts of the plants fluctuated in accordance with the different kind of the crops and the application methods of ^{134}Cs, accompanying the general trend of the lowest value in stem and sheath, and the higher value in brown rice as compared with those in husk, rachis and upper leaf.

Abnormally low $^{134}Cs/K$ ratio in lower leaves of the top dressing pot was presumably due to the difficult redistribution of ^{134}Cs from older leaves.

Along with the high ^{134}Cs contents and $^{134}Cs/K$ ratios, lowland rice absorbed extremely high amounts of ^{134}Cs applied to the soil as compared with upland rice. The percentages of ^{134}Cs absorbed by plants against the application dose were 0.56–1.22 for lowland rice and 0.033–0.049 for upland rice, decreasing in the following order in relation to the application methods of ^{134}Cs;

Top dressing $>$ basic mixed placement $>$ basic surface application

As for $^{134}Cs/K$ ratios, lowland rice indicated about ten times the values of uplane rice.

According to the analysis of air dry soils, the high fixing ability of ^{134}Cs by the soil and the excellent selective absorption of K against ^{134}Cs by plant roots were observed distinctly.

These results would suggest the unusual behaviour of the trace amounts of cesium in the system of water-logged soil and lowland rice.

51. **Radioactivation analysis of strontium and barium in soil and plant.** Shingo MITSUI, Kiyoshi TENSHO and Ko-Ling YEH (Journ. Sci. Soil Manure, Jap. 30, 353–356, 1959)

Using the technique of radioactivation analysis, the determination of Sr and Ba was made with regard to various plant portions of carrot and lowland rice along with exchangeable bases of the pertinent soils.

The results obtained with Ca, Sr and Ba in soil and plant are as follows.

1) Ba content of soils was 2–3 times higher than that of Sr. On weight basis and in terms of the concentration against Ca, 0.34% and 0.40% were obtained for Sr as compared with 0.72% and 1.32% for Ba respectively.

2) On dry matter basis, Sr content of carrot was higher than that of Ba, but the reverse relationship was shown in almost all parts of lowland rice except leaf blade.

Sr/Ca and Ba/Ca of carrot remained almost constant between leaf and root, but for lowland rice this ratio of brown rice and husk was higher than that of leaf blade and stem.

This tendency was more significant in the case of Ba, resulting from the remarkable high accumulation of this element in the ear.

3) Regarding Sr/Ca, there was no significant difference between soil and plant. As for Ba/Ca ratio, the value, in the case of carrot, was about one fifth in comparison with the soil showing the remarkable selective absorption of Ca against Ba.

In the case of lowland rice, however, this selective absorption was not clear, and the preferential absorption of Ba was shown as compared with carrot, presumably due to the increasing exchangeability of this element under water-logged soil conditions.

52. **Effect of cobalt-60 gamma radiation on urea and urea containing fertilizers.** Shingo MITSUI and Hidehiko NISHIMURA (Journ. Sci. Soil Manure, Jap. 30, 511–514, 1960)

The effect of cobalt-60 gamma radiation on urea and urea derivatives was determined by ultrared spectrum analysis, gas analysis and by incubation experiment in the soil.

As for urea H_2, CO_2, and CO were detected as decomposition products. The evolution of CO, however, needs further study.

As for urea compound fertilizers a considerable change was observed by ultrared spectrum analysis, indicating a possible combination of urea with other components.

A slight decomposition was observed in $U/F=5$, one of the urea and formaline condensation products. However, in the incubation experiment in the soil no change could be observed.

Some discussions were made on the amount of radiation and the temperature at the time of radiation, but due to the scarcity of available data it needs further study.

53. **Nutritional study of silicon in graminaceous crops. I. Growth of the rice plants without SiO_2 and symptoms of SiO_2 deficiency.** Shingo MITSUI and Hiroshi TAKATOH (Journ. Sci. Soil Manure, Jap. 30, 535–539, 1960)

Rice plants were grown in solution culture in polyethylene pots in the biotron and green house. Nutrients salts were chemical pure. All water used was distilled in a tin-lined copper still (0.005 ppm SiO_2 in water). The SiO_2 treatments were without SiO_2 plots, 20 ppm SiO_2 plots and 100 ppm SiO_2 plots.

The results obtained are as follows:

1) At the early stage of plants growth, necrosis of older leaves was observed as symptoms of SiO_2 deficiency.

2) The mean yield (weight of total dry matter) of all plants supplied with additional

100 ppm SiO_2, was about 70% greater than the mean yield of the plants without SiO_2 treatment and the same in the percentages of ripened grains.

3) The heading date of the rice plants of SiO_2 plot (add. 100 ppm SiO_2) was earlier by 4-10 days than that of without SiO_2 plot.

4) Effect of checking contamination of Si-dust on the foliage was recognized, in the biotron conditions.

It was concluded that Si would be essential for the growth of the rice plant.

54. **Effect of light and nutritional conditions of rice plant on the appearance of asparagine in leaves at the early panicle formation.** Shingo MITSUI, Kikuo KUMAZAWA and Katsumi ONO (Journ. Sci. Soil Manure, Jap. 30, 433-438, 1959)

Effect of light intensity and nutritional conditions, particularly of nitrogen, phosphorus and potassium on the appearance of asparagine in rice leaves at the early panicle formation was investigated in the present experiment. Asparagine appearance was determined with the help of simplified paperchromatography technique, and the colour density of leaves was graded only by observation.

The results obtained are summarised as follows:

The results were: 1. In low light intensity condition the colour density of leaves increased whereas asparagine did not. 2. In high nitrogen, both colour density of leaves and asparagine increased. 3. By the addition of potassium both asparagine and colour density decreased.

From above results and amino acid content at the period of early panicle formation and yields of straw and grain, it can be concluded that the asparagine was produced by the process of protein decomposition accompanied with the turning of growth phase or irregularity of it.

It becomes very apparent that the method of determining the necessity nitrogen top dressing at the early panicle formation for rice by the appearance of asparagine in the leaves was quite useful under the various conditions.

55. **Asparagine test in relation with the nitrogen nutritional status of crop plants. Part 2. Barley.** Mahatim SINGH, Kikuo KUMAZAWA and Shingo MITSUI (Soil and Plant Food, 5, 101-107, 1959)

In the previous paper we have concluded that the appearance of asparagine in the rice leaves is the resultant effect of nitrogen levels and light intensities and also that its appearance may be taken as a basis of top dressing. The present experiment was conducted to know whether asparagine appears in the barley leaves and whether it is governed by similar factors as in the case of rice. Since barley is an upland crop, in addition to asparagine, nitrate was determined and the following results were obtained:

1) Darkness with increasing duration has opposite effect on the appearance of nitrate and asparagine in the leaves and stem. The content of nitrate decreases whereas that of asparagine increases irrespective of stage of leaves and nitrogen levels.

2) The decrease of nitrate is very rapid whereas the increase in asparagine is rather gradual.

3) The high level of nitrogen responds in one way as the high concentration of asparagine in leaves and stem with increasing duration of darkness and on the other hand controls the rapid decrease of nitrate in comparison with low level of nitrogen.

4) Young leaves behave differently in comparison to stem and old leaves regarding the

appearance of nitrate and asparagine. The content of nitrate in the young leaves remains lower to stem and old leaves even with the increasing darkness and its duration, but the content of asparagine remains always higher and increases with the duration of darkness.

5) In the early period of light treatment nitrate increased in the old leaves and stem but it generally decreased from the very beginning in the young leaves.

6) After top dressing, the content of nitrate increased in general, but the plants which were under low level of nitrogen and complete darkness showed rapid increase of nitrate. The content of asparagine decreased particularly in the plants which were under complete darkness and low nitrogen. After top dressing asparagine appeared much more in the young leaves, stem and less in old leaves but nitrate appeared much more in the old leaves and stem than young leaves. On very remarkable trend of asparagine was observed that it appeared in all parts again after its absence, but not in the case of nitrate.

Part 3. Rice. Mahatim SINGH, Kikuo KUMAZAWA and Shingo MITSUI (Soil and Plant Food, 5, 167–173, 1960)

In the previous investigation it has already been found that the asparagine appearance is the resultant effect of panicle formation initiation whereby decomposition of proteinaceous compounds takes place. Also, the appearance of asparagine is influenced by the presence or absence of nitrogen source and by the stage of the plant parts. The present experiment was designed to investigate the role of different forms of nitrogen towards the appearance of asparagine and to study the appearance of asparagine in relation with the nitrate appearance throughout the life cycle of the crop. The present report embodies results of the above studies as follows:

1) Nitrate appearance was detected irrespective of nitrogen levels in the early stage of the crop, but its appearance was limited in the roots and stem. It also appeared extensively in the roots, stem and almost all the leaves 10 days after the top dressing of sodium nitrate and ammonium nitrate.

2) Asparagine appearance was detected in the case of high nitrogen, either from the very beginning or from the top dressing, but in the case of low nitrogen only around the panicle formation or afterwards.

3) All the leaves are interrelated as regards the appearance of asparagine. The general trend of its appearance is that a decrease in one leaf which has gone old is accompanied by a corresponding increase in the younger leaves. It indicates that the asparagine is one form of translocation of nitrogenous compounds after the initiation of panicle formation from one part to the other.

4) The appearance of asparagine is directly influenced by the ammonium form of nitrogen. The nitrate nitrogen has only an indirect and long term influence over the asparagine appearance.

5) The urea form of nitrogen is only intermediate to ammonium and nitrate nitrogen.

6) The asparagine test holds good as compared to nitrate test for determining the top dressing requirement of crop plants.

56. Estimation of number of root nodule bacteria by nodulation-dilution frequency method and some applications. (Ecological studies of *Rhizobium* in soils. Part 1.) Katsura TSUZIMURA and Iwo WATANABE (Journ. Sci. Soil Manure, Jap. 30, 292–296, 1959)

The dilution frequency method based on the nodulation on the host plant was studied to estimate the number of root nodule bacteria in soils. Application of this method to the

pure culture suspensions or to the suspensions mixed with soil showed fairly good agreement with the plate count method, with a few unexplainable exceptions.

By this method the authors made surveys on the distribution of the nodule bacteria of "genge" (*Astragalus sinicus*) in a experimental field for rotation. The field was tenth year (20th crop) of the rotation. The summer crop was rice in common. The winter crops were wheat and genge. About 10^5 genge-bacteria per 1 g of dry soil were found in the soils of the rice-wheat-rice-genge rotation plots regardless of the standing crops. Only 10^1 of genge-bacteria were counted in the adjoining rice-wheat plots. Other micro-flora was not significantly different between the plots. It was clear that the cropping of the host plant exerted distinct influence on the number of root nodule bacteria in the soil. Genge-bacteria survived under water-logged conditions in summer.

57. **Saprophytic life of *Rhizobium* in soils free from the host plant. (Ecological studies of *Rhizobium* in soils. Part 2.)** Katsura TUZIMURA and Iwao WATANABE (Journ. Sci. Soil Manure, Jap. 30, 506–510, 1960)

The authors surveyed the population of root-nodule bacteria in field soils in relation to the cultivation of the host crops. 1,000–16,000 alfalfa bacteria (*Rh. meliloti*) per gram soil were counted in field under standing alfalfa, and 1,000–2,500 in the field 2 years after the removal of alfalfa. The number of "genge" bacteria (*Rh.* sp.) were 2,000–7,000 in fields cultivated in the spring of the year, while about 20–60 "genge" bacteria were in orchard soils, where "genge" (*Astragalus sinicus*; Chinese mild vetch) had not been cropped since 5–10 years before.

Numbers of soybean bacteria (*Rh. japonicum*) were 50,000 and 3,000 in parts of a field cropped with soybean one and two years before respectively. In a adjoining part of the field where soybean was not cropped at least for 20 years, the number was less than 4. But in neighboring forest soil 100 soybean bacteria were counted per gram soil. Existence of the wild host plant in the forest was not checked.

"Genge" bacteria again increased when air-dried and partially sterilized soil was rewetted. This showed the ability of *Rhizobium* to multiply saprophytically in competition with other soil micro-organisms.

Although density of *Rhizobium* is the highest under standing host crops, they survive as a saprophytic bacteria in soils without the hosts, and decrease gradually as time proceeds.

58. **Multiplication of *Rhizobium* in the rhizosphere of host plants. (Ecological studies of *Rhizobium* in soils. Part 3.)** Katsura TUZIMURA and Iwao WATANABE (Journ. Sci. Soil Manure, Jap. 31, 173–176, 1960)

The author studied the effect of host plant roots on the population of Rizobia in the surroundings of roots. The rhizosphere of genge plant (*Astragalus sinicus*) supported more abundant genge rhizobia than soil remote from host roots. The effect was equally ascertained when rhizobia were inoculated on the soil that was free from rhizobia, or seed was sown on the soil where rhizobia were established. In both soil examined, the population of rhizobia in rhizosphere was almost equal. Marked increase of the population in rhizosphere was observed at the stage of fruiting, reasonably due to the out flow of rhizobia from the generated nodules.

Multiplication of rhizobia affords the reasonable basis for seed inoculation practice.

59. **Respiration substrate of *Rhizobium* in the nodules.** Katsura TUZIMURA and Hiroshi MEGURO (Journ. Biochem. 47, 391–397, 1960)

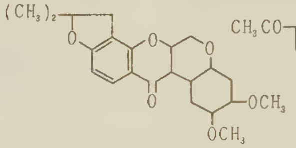
Three different cells of *Rhizobium japonicum*, viz. the cells grown on glucose, the cells grown on succinate, and the cells taken from soybean nodules, were compared with respect to the ability to oxidize substrates, including carbohydrates, the intermediates of glycolysis and the organic acids of Krebs cycle.

Organic acids were readily oxidized by all the three types of the cells. Among the hexose and triose-phosphates, only fructose-1, 6-diphosphate was oxidized readily. Glucose was oxidized readily only by cells cultured on glucose, and slowly by the cells culture on succinate, but not by the symbiotic cells. The symbiotic cells did not oxidize any of glucose, fructose, sucrose and mannitol. These facts suggest that the energy sources of *Rhizobium* in symbiotic state are organic acids but not carbohydrates, although it is possible that fructose-1, 6-diphosphate is also used.

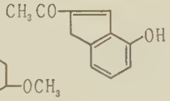
60. Synthesis and configurational elucidations of rotenoids. Part 11. Synthesis of dihydrorotenone. Masateru MIYANO and Masanao MATSUI (Chem. Ber. 92, 2487–2492, 1959)

The first total synthesis of 1-dihydrorotenone was reported in part 10 as short communication. Detailed descriptions are given in this part.

Starting from 2-acetyl-4-hydroxy-cumaron [II], which was synthesized from resarcin in 6 steps, roteol [III] is obtained in 3 or 4 steps. 1-dihydrotubanol [IV] has been known to be obtainable from [III] in 5 steps. By Hoesch condensation the former reacted with a nitril [V] and after saponification we obtained dihydroderrisic acid [VI]. As we have already succeeded in a 3 step partial synthesis of 1-dihydrorotenone from [VI], this report means 17-step total synthesis of 1-dihydrorotenone.



[I]



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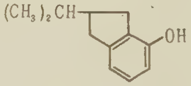

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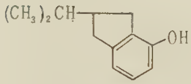

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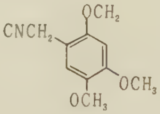

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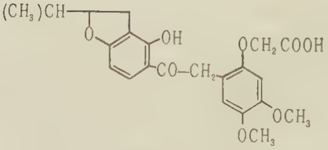


[VI]

Part 12. Synthesis of d,l-nordihydrorotenones. Masateru* MIYANO and Masanao MATSUI (Chem. Ber. 93, 54–61, 1960)

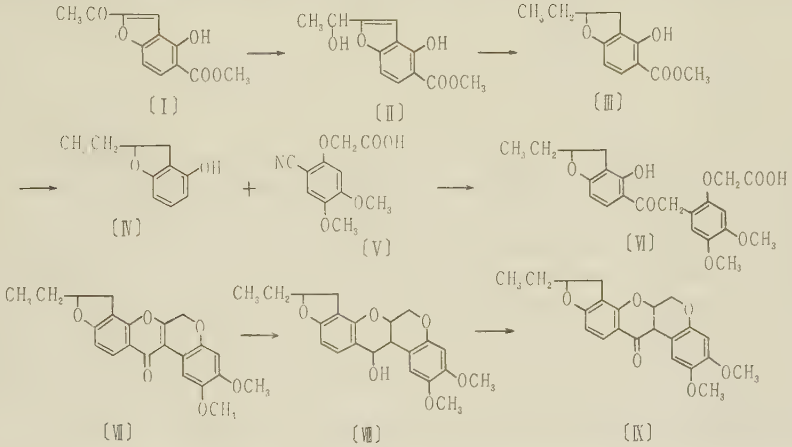
2-Acetyl 4-hydroxy 5-carbomethoxycumarone [I] is reduced to 2-[α-hydroxyethyl]-4-hydroxy-5-carbomethoxycumarone [II] in the presence of palladium catalyst. Catalytic hydrogenation of [II] with platinum dioxide in pure acetic acid gives methyl ester of

d,1-nordihydrotubaic acid [III]. After hydrolysis and decarboxylaction of [III] is obtained a key—intermediate of this synthesis, nordihydrotubanol [IV], in good yield based on the starting material [I].

By Hoesch condensation of [IV] with nitril [V] we can obtain d,1-nordihydro derrisic acid [VI] in 22% yield. Treating with acetic anhydride the latter is cyclized and yields d,1-nordihydrodehydrorotenone [VII] in 37% yield. I.R. spectra of [VI] and [VII] in chloroform are shown to be identical with those of natural products.

By reduction of [VII] with sodium borohydride in dioxan d,1-nordihydro rotenol [VIII] is obtained.

Oppennauer oxidation of this racemate followed by purification with chromatography gives d,1-trans nordihydrorotenone as colourless crystal, mp 150-152°, in 15.7% yield. In comparison with I.R. spectra it is shown that [IX] has same configuration about asymmetric carbon atoms as natural 1-dihydrorotenone.



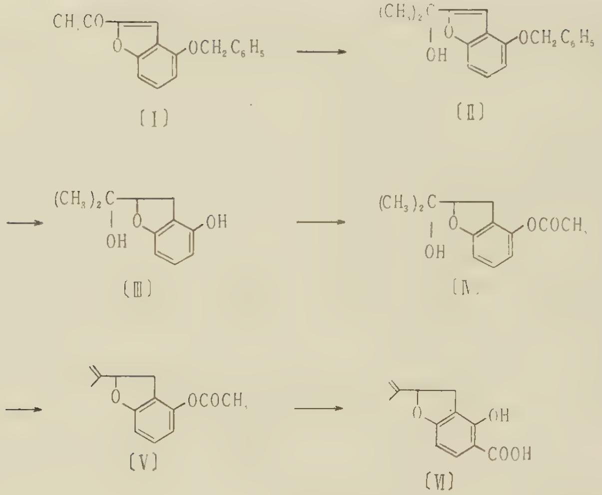
Part 13. Synthesis of tubaic acid. Masateru MIYANO and Masanao MATSUI (Bull. Agr. Chem. Soc. Jap. 24, 218-219, 1960)

This paper is a short communication to the editors of Bull. Agr. Chem. Soc. Japan. Detailed descriptions are given in part 14 together with other new facts.

Part 14. Synthesis of tubaic acid and related cumarones. Masateru MIYANO and Masanao MATSUI (Chem. Ber. 93, 1194-1201, 1960)

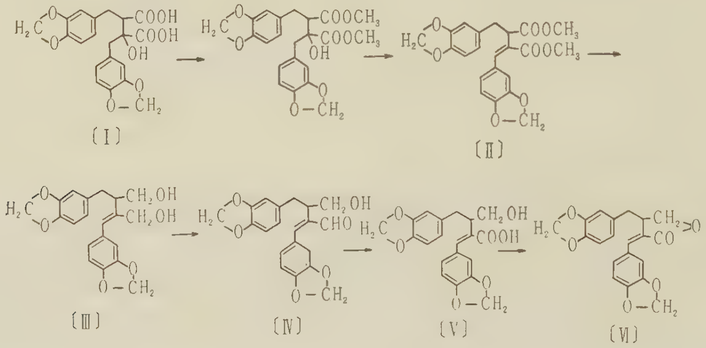
The reaction of 4-benzyloxy-2-acetyl-cumarone [I] with methyl magnesium iodide gives 4-benzyloxy 2-[α-hydroxy isopropyl] cumarone [II], which is reduced in the presence of Raney Nickel to d,1-hydroxydihydrotubanol [III], mp 150-151°, in good yield (75% based on [I]). Acetylation of [III] with acetic anhydride-pyridine yields monoacetate [IV], which is dehydrated to d,1-tubanolacetate [V] with phosphorus tribromide in pyridine at 0°C. This racemic compound is also obtained from the mixture of [IV] and methane sulfochloride in pyridine under 0°C.

The sodium salt of d,1-tubanol is dissolved in ethyleneglycol and carbondioxide is introduced at high temperature. This acid thus obtained, mp 187-188°, shows identical by I.R. spectra with that of natural 1-tubaic acid [VI] in chloroform. The synthetic d,1-mixture forms brucine salts and 1-tubaic acid is divided as ethanol insoluble brucine salt.



61. Studies on phenolic lactones. Part III. Synthesis of (±) hibalactone. Kyohei YAMASHITA and Masanao MATSUI (Bull. Agr. Chem. Soc. Jap. 24, 160–162, 1960)

(±) Hibalactone, the racemic form of α-piperonylidene-β-piperonyl-butyrolactone, was synthesized by the following method. α-piperonylidene-β-piperonyl-succinic acid dimethyl ester [II], m.p. 87–88°, resulted from the methylation and dehydration of α-(3,4-methylenedioxyphenyl-hydroxymethyl)-β-(3,4-methylenedioxybenzyl)-succinic acid [I], was reduced with lithium aluminum hydride to 2-piperonylidene-3-piperonyl-1,4-butanediol [III], m.p. 117°. Oxidation of the glycol with manganese dioxide in acetone yielded α-piperonylidene-β-piperonyl-γ-hydroxybutyr aldehyde [IV] which afforded racemic hibalactonic acid [V], m.p. 143–144°, after oxidation with silver oxide. Lactonization of hibalactonic acid by warming with dilute hydrochloric acid afforded (±) hibalactone [VI], m.p. 154–155°.



62. Biosynthesis of carotene in disks of carrot root. Keiji HARASHIMA (Bull. Agr. Chem. Soc. Jap. 24, 203–210, 1960)

Disks obtained from carrot roots were cultured *in vitro*, and carotene contents in these disks were measured at various time intervals. Net synthesis of carotene was demonstrated,

when the disks were grown on media containing fructose, glucose or sucrose. Mannitol showed no effect on both growth of the disks and carotene synthesis. Diphenylamine inhibited the synthesis of carotene in the disks in a way somewhat different from that in the case of microorganisms. Although yeast extract stimulated growth of the disks, it showed no effect on carotene synthesis. Addition of possible precursors for carotene synthesis to the medium showed no stimulatory effect except when β-hydroxy-β-methylglutaric acid was added to the medium rich in sucrose.

63. **Isolation of 2-aminoethane phosphonic acid from rumen protozoa.** Masaaki HORIGUCHI and Makoto KANDATSU (Nature, 184, 901–902, 1959)

In the course of study on the amino acid composition of sheep rumen protozoa, an unknown ninhydrin-positive substance was found by paper chromatography to be present in acid hydrolysates of ether-ethanol soluble fraction of protozoa. The substance was isolated in crystalline form and identified as 2-aminoethane phosphonic acid, $H_2N \cdot CH_2 \cdot CH_2 \cdot PO(OH)_2$, and the occurrence of a C-P bond in nature has been demonstrated for the first time.

64. **Studies on the nutritive value of grass proteins. Part 8. The effect of drying methods upon the digestibility of soy bean leaf proteins. (3). The effect of drying method with aeration at 40-50°.** Makoto KANDATSU and Tadahiko YASUI (Journ. Agr. Chem. Soc. Jap. 33, 893–898, 1959)

The changes in some constituents of soy bean leaf dried with aeration at low temperature were followed. The loss of crude protein during drying was small and the changes of the other constituents were almost none. The digestibilities of protein and nitrogen free extracts were lowered about 2 and 5 per cent respectively by drying, but digestibility coefficient of crude fiber was higher than that of the fresh leaf and those of the others were about 3–5 per cent lower than those of the fresh one. According to drying, water soluble, 10% NaCl soluble and 0.3% NaOH soluble proteins decreased remarkably and insoluble proteins increased very much. At the same time, the apparent digestibilities of the former three proteins were lowered and that of the latter was risen. From the results of our experiment, it was suggested that the decrease of apparent digestibility of protein may be caused by considerable increase of metabolic fecal nitrogen excretion in the feces.

(4). **The effect of drying in the sun.** Makoto KANDATSU and Tadahiko YASUI (Journ. Agr. Chem. Soc. Jap. 33, 899–903, 1959)

The changes in some constituents of soy bean leaf dried in the sun were followed. The nitrogen free extracts were much, but the others were slightly lost. Nevertheless, the distribution of proteins in the leaf underwent remarkable changes, that is, 0.3% NaOH soluble protein increased remarkably, whereas the water soluble, 10% NaCl soluble and insoluble proteins decreased.

Although apparent digestibility coefficient of crude fiber was even higher than that of fresh one, those of protein and nitrogen free extracts were lowered as compared with those of fresh leaf and dried leaf with aeration at low temperature, especially, those of 0.3% NaOH soluble, water soluble and insoluble proteins were lowered, whereas that of 0.3% NaOH 60% hot alcohol soluble protein did not change.

It was suggested from the results obtained that the decrease of apparent digestibility of protein may be caused not only by the changes of native proteins into undigestible ones owing to reaction with carbohydrate during drying, but also by the considerable increase of metabolic fecal nitrogen excretion in the feces.

65. Studies on reticulo-rumen digestion. XVI. Cultivation of infusoria. Part 4. Makoto KANDATSU and Naomi TAKAHASHI (Jap. Journ. Zootech. Sci. 30, 166–170, 1959)

Rumen protozoa which inhabit the reticulo-rumen of the sheep and goat were cultivated in vitro at 39°C by using culture media containing amino-acid mixture, peptone, whole egg, soy-bean extract, and yeast extract in place of rumen bacteria.

Infusoria, to which *Entodinium* belongs, were alive in media containing these nitrogen compounds. Especially in media which contained yeast extract, infusoria were active in motile state and survived almost the same length of time as they did in media to which hay extract and rumen bacteria were added.

It was observed that in media through which CO_2 or H_2 gas had passed, infusoria became more active. As for the effect of yeast extract, it was clarified that its effective factor consisted in two fractions obtained from acetone treatment, one being the extracted fraction and the other the precipitated. The latter fraction could be substituted by peptone or amino-acid mixture. Similar results were obtained from experiments on the effectiveness of rumen bacteria.

In culture media which were prepared from these substances, it was observed that infusoria were active and that frequent fission took place only when some amount of clover juice was added to the media. Clover juice was effective to cause fission among infusoria grown in media which contained no rumen bacteria.

66. On the nutritive value of the yeast protein. Part II. Makoto KANDATSU and Yoko SAITO (Food and Nutrition, 12, 76–78, 1959)

The nutritive value, especially biological value, of the protein of dry yeast (*Mycotorula japonica*) grown on sulfite waste liquor of wood was studied and the following results were obtained.

The total crude protein of the yeast was about 53% in dry basis. The successive extractions were carried out by water, dilute alkali, and hot dilute alkali alcohol etc.. Taking the amount of total crude protein as 100, that of total extractable protein was 95. In which, the amount of water extractable protein was 13, and those of dilute alkali and hot dilute alkali alcohol were 28 and 47, resp..

The biological values of the yeast protein and the whole egg protein determined with rats were 70 and 100, resp. at the 5% level of protein intake, and 64 and 79, resp. at its 10% level, and their digestibilities were 84 and 92%, resp..

Concerning the biological value, the yeast protein is nearly equal to the high nutritional plant protein, such as oat, corn, and potato, but is inferior to animal proteins.

Part III. Makoto KANDATSU and Yoko SAITO (Food and Nutrition, 12, 334–337, 1960)

In order to have precise knowledge on the nutritive value of yeast protein, the rats were fed the diets containing 42% and 90% of yeast grown on sulfite waste liquor of wood, and urinary allantoin-nitrogen and uric acid-nitrogen were estimated. These values were 50–70 γ/mg intake N and 1–3 γ/mg intake N, resp. and were about 5 times as much as those from rats on dry whole egg feeding.

It is thought that the biological value of about 6% of total N of the yeast is zero, since about 6% of total N of the yeast is excreted in the form of allantoin-nitrogen in urine, so the biological values of the yeast protein are calculated 75 at 5% level of protein intake and 68 at 10% level of protein intake from the data in the Part II of this report. These values are similar to those of animal proteins.

67. Chemical studies on actin. Part 1. On the changes of bindings of action to alkaline earth metals during aging of meats. Masao FUJIMAKI, Nobuhiko ARAKAWA and Hiroko YONETA (Bull. Agr. Chem. Soc. Jap. 24, 333–337, 1960)

The content of alkaline earth metals in actin during aging of meats was determined. The content in actin was found to be maximum immediately after slaughter and minimum at two days of aging. The rate of calcium to alkaline earth metals except calcium was high immediately after slaughter, but became low during aging.

As the content of alkaline earth metals in actin extracted from the acetone dried actomyosin has been found to be less than that from the same muscle, the result obtained appears to show that in meats actin exists in both free and bound forms, the latter being one constituent of actomyosin, and at the process of aging in which minimum content of alkaline earth metals in actin was observed, actin was more in the form of actomyosin than at the other process.

68. On the reaction of cooked and stored meats with 2-thiobarbituric acid (TBA). Part 1. On the changes of TBA values of cooked meats during storage. Masao FUJIMAKI and Fujiko YOSHIMATSU (Journ. Agr. Chem. Soc. Jap. 34, 518–523, 1960)

Cooked and stored meat showed much increased 2-thiobarbituric acid (TBA) values, compared with raw meat or cooked and stored meat added with polyphosphate.

TBA values and peroxide numbers of fats extracted from cooked and stored meats by ether as a solvent showed little change, holding low levels, but TBA values of defatted cooked and stored meats showed much increase during the storage of two weeks.

The reacting substances with TBA reagent in cooked and stored meats were little extracted by such an organic solvent as ether, chloroform or ethanol, but most parts of them were found to be extracted by trichloroacetic acid solution.

69. Studies on browning reactions between sugars and amino compounds. Part IV. Identification of 5-hydroxymethylfurfural formed from aromatic-amine-N-hexosides and its role in the browning reaction. Hiromichi KATO (Bull. Agr. Chem. Soc. Jap. 23, 551–554, 1959)

N-D-glucosyl (or galactosyl)-p-aminobenzoic acid has been found to form melanoidins in methanol solution acidified with hydrogen chloride at 25° for twenty-four hours. From the reaction mixture 5-hydroxymethylfurfural (HMF) has been isolated and identified as its, 2, 4-dinitrophenylhydrazone in the yield of 7.5% of the theory.

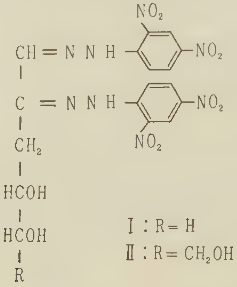
So, comparisons between the browning reaction of HMF or furfural with aromatic amine and that of the corresponding N-glycosides have been made under the same condition. From the results obtained, it has been shown that, under the described condition, furfural is almost inactive for browning, while on the contrary, HMF is active and plays an important role in the browning reaction.

Part V. Isolation and characterization of new carbonyl compounds, 3-deoxyosones formed from N-glycosides and their significance for browning reaction. Hiromichi KATO (Bull. Agr. Chem. Soc. Jap. 24, 1–12, 1960)

Both aliphatic and aromatic amine-N-pentosides decompose to form melanoidins by weak acid catalysis. From their brown reaction mixture, an unknown carbonyl compound, $C_{17}H_{16}N_8O_{10}$, bas been isolated as its bis-2,4-dinitrophenylhydrazone. And, similarly from the reaction mixture of either aliphatic or aromatic amine-N-D-glucoside, another unknown hydrazone, $C_{18}H_{18}N_8O_{11}$, has been isolated. As the results of characterization, it has been proved that the former is 3-deoxy-xylosone bis-2,4-dinitrophenylhydrazone (I) and the latter

is 3-deoxy-D-glucosone bis-2,4-dinitrophenylhydrazone (II).

It has been shown that 3-deoxy-osone is the intermediate in the melanoidin formation. Furthermore, its significance in the browning reaction is pointed out.



70. Mechanism of cyanide resistance in *Achromobacter*. I. Adaptive formation of cyanide resistant respiratory system in growing cells. Shôji MIZUSHIMA and Kei ARIMA (Journ. Biochem. 47, 351–360, 1960)

On strain of *Achromobacter* isolated from soil was found to show a cyanide resistant growth adaptively in the presence of 10^{-3} *M* of cyanide.

The respiration of the cyanide adapted cells was cyanide insensitive, whereas that of the cyanide non-adapted cells was remarkably inhibited by cyanide.

The oxidation of DPNH and succinate by the cell-free preparations obtained from the cyanide non-adapted cells was remarkably inhibited by cyanide, whereas that obtained from the adapted cells was less inhibited.

The DPNH or succinate oxidase activity was almost located in the subcellular particulate fraction, and the terminal oxidase was found to be a one electron carrier.

Cytochromes b_1, a_1 and a_2 were found in the particulate fraction in both the cyanide adapted and the non-adapted cells. However, the amounts of cytochrome a_2 (and a_1) were greatly increased during the adaptation to cyanide.

It was postulated that the mechanism of cyanide resistance in the bacteria was caused by the increase of cytochrome a_2 (or a_1) which may be strongly inhibited by cyanide in nature. This is a new type of the mechanism in the drug resistance.

II. Adaptive formation of cyanide resistant respiratory system in resting cells. Shôji MIZUSHIMA and Kei ARIMA (Journ. Biochem. 47, 600–607, 1960)

It was demonstrated that the respiratory system of a strain of *Achromobacter* could adaptively become cyanide resistant without bacterial growth. During the adaptation, the amount of cytochrome a_2 in resting cell remarkably increased as in the case of the adaptation in growing bacteria. Cyanide, energy source and amino acids were required for the adaption. The optimal concentration of cyanide in tne medium was $1\text{–}2\times10^{-3}$ *M*.

Streptomycin (10^{-3} *M*) inhibited the bacterial growth but did not inhibit the adaptation to cyanide. Chloramphenicol (10^{-5} *M*) only inhibited the adaptation to cyanide but did not inhibit the bacterial growth in bouillon medium. The mechanism of the adaptation was discussed especially with regard to the mode of action of chloramphenicol.

III. Nature of terminal electron transport system and its sensitivity to cyanide. Shôji MIZUSHIMA and Kei ARIMA (Journ. Biochem. 47, 837–845, 1960)

Water soluble preparation which catalyzes the electron transfer between DPNH or succinate and oxygen was obtained from subcellular particulate preparation of a strain of *Achromobacter*. Cytochromes b_1 and a_2 contained in the preparation were slowly reduced by succinate or DPNH and oxidized in an instant by oxygen. Cyanide (10^{-3} M) remarkably but not completely inhibited the oxido-reduction of cytochrome a_2 but did not inhibit the reduction of cytochrome b_1. The direction of electron transport between succinate or DPNH and oxygen was postulated as follows.

DPNH-dehydrogenase ╲
succinate-dehydrogenase ╱ cytochrome b_1—a_2—oxygen

These results supported a view on the mechanism of cyanide resistance in this bacteria.

71. Metabolism of aromatic compounds by microbes. Part VI. Existence of two pathways, the pathway *via* protocatechuic acid and the one *via* gentisic acid, in the metabolism of *m*-hydroxybenzoic acid by bacteria. Shin-ichi SUGIYAMA, Keiji YANO, Kazuo KOMAGATA Masaji KAZAWA and Kei ARIMA (Bull. Agr. Chem. Soc. Jap. 24, 211–216, 1960)

Oxidation of *m*-hydroxybenzoic acid by eleven bacterial strains which were able to metabolize this compounds as a sole source of carbon was carried out. The cultural broths were examined by paperchromatography for detection of intermediates.

Gentisic acid was identified in the culture filtrate of the five strains, while protocatechuic acid by the remaining six strains. Protocatechuic acid was not detected in all the cultural broth of bacteria which belong to the gentisic acid path type, and *vice versa*.

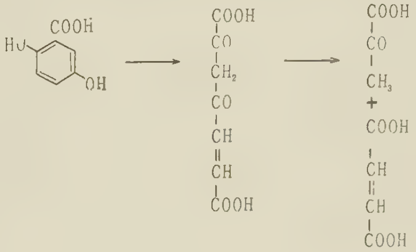
Strains which posses the gentisic acid path were *Pseudomonas ovalis* (two strains), *Micrococcus ureae*, and an unidentified *Bacterium* and a strain of *Aerobacter*. Strains which have the protocatechuic acid path were *Pseudomonas dacunhae* (five strains) and *Pseudomonas cruciviae*.

An unknown keto acid was detected as its 2,4-dinitrophenylhydrazone from the incubation mixture of the resting cells of *Pseudomonas ovalis* S-5 and gentisic acid. This new keto acid was most probably the direct intermediate after the cleavage of gentisic acid.

Part VII. Gentisic acid oxidase. Shin-ichi SUGIYAMA, Keiji YANO, Kazuo KOMAGATA and Kei ARIMA (Bull. Agr. Chem. Soc. Jap. 24, 243–248, 1960)

Cell-free enzyme preparation which catalyzes an oxidative breakdown of gentisic acid with rupturing of benzene ring was obtained from *m*-hydroxybenzoic acid induced cells of *Pseudomonas ovalis* S-5. This new benzene ring splitting enzyme was named "Gentisic acid oxidase".

Comparison of various methods to obtain a cell-free enzyme preparation were made. The enzyme was partially purified, and ferrous ion was proved necessary, for the activity of this



enzyme. Further, some other properties of the gentisic acid oxidase were also investigated.

From the various evidences obtained by manometrical experiments and chemical reaction of the product of this enzyme, the oxidative pathway of gentisic acid by *Ps. ovalis* S-5 was presumed to be as above mentioned.

Part VIII. Further studies on gentisic acid oxidase. Shin-ichi SUGIYAMA, Keiji YANO and Kei ARIMA (Bull. Agr. Chem. Soc. Jap. 24, 249–254, 1960)

A highly purified gentisic acid oxidase preparation free from protocatechuic acid oxidase was obtained by the repeated ammonium sulfate precipitations. The specific activity increased up to twenty-fold. This enzyme preparation is free from oxidases of other aromatic compounds, as maleylpyruvate isomerase and fumarylpyruvate hydrolyase.

The activity of this enzyme preparation was remarkably inhibited by metal reagents such as α,α'-bipyridyl, ethylenediaminetetraacetic acid, *o*-phenanthroline and nitroso-R salt, and the inhibition was reversed by the addition of ferrous ions, and reactivation increased by further addition of reducing agents, but not by the reducing agent such as ascorbic acid alone.

Enzyme and ferrous ion combination was proved to be necessary for the activity of the gentisic acid oxidase as the results of experiments using several combination of iron-free enzyme, substrate and ferrous ions.

The loss of activity of iron-free enzyme could be recovered by the addition of ferrous ion. Other metalic ions and reducing agents tested were not effective.

Participation of SH-radical in the enzyme-substrate combination was presumed from the inhibition pattern by SH-reagents such as *p*-chloromercury-benzoate.

Part IX. The enzymatic conversion of genetisic acid to fumarylpyruvic acid. Shin-ichi SUGIYAMA, Hirosato TANAKA, Keiji YANO and Kei ARIMA (Bull. Agr. Chem. Soc. Jap. 24, 255–261, 1960)

One of the presumed intermediates of the gentisic acid oxidation was obtained in a crystalline form using dialyzed crude gentisic acid oxidase, free from fumarylpyruvic acid hydrolyase, from the cells of *Pseudomonas ovalis* S-5.

From the results obtained, the crystalline intermediate was identified as fumarylpyruvic acid, which was splitted by crude extracts into pyruvic and fumaric acids.

Maleylpyruvic acid, the initial product of gentisic acid oxidation, was recognized spectrophotometrically in the reaction mixture using purified enzyme. And the pathway of gentisic acid oxidative metabolism was established.

72. Studies on the distribution and properties of lactic acid bacteria in cow's milk in Japan. VII. Acid-producing capacity and acidoduric property of lactic acid bacteria. Rinjiro SASAKI and Toshitaka NAKAE (Jap. Journ. Zootech. Sci. 30, 134–137, 1959)

Acid-producing capacity and acidoduric property were investigated on 256 strains of lactic acid bacteria which had been isolated from raw milk in the previous study of this series. A strain of *Streptococcus faecalis* was able to coagulate skim milk within 4 hours of incubation. Streptococci generally showed a higher acid-producing rate than lactobacilli.

The highest acidity produced by streptococci in skim milk during 15 days of incubation was 1.62% and given by *Str. lactis* D-10 and that lactobacilli was 2.15% and given by *Lactobacillus casei* A-7.

Many acidoduric strains were found when the organisms of *Leuconostoc dextranicum*, *Lb. plantarum*, *Lb. casei*, and *Str. faecalis* were tested.

VIII. Aroma-producing property of lactic acid bacteria. Rinjiro SASAKI and Toshitaka NAKAE (Jap. Journ. Zootech. Sci. **30**, 138–140, 1959)

The 256 strains of lactic acid bacteria, which were isolated from raw milk in the previous studies, were examined for properties to produce such aromatic compounds as diacetyl and acetoin. As a result, some strains of *Streptococcus faecalis*, *Str. lactis*, and *Lactobacillus casei* were found to have relatively high aroma-producing abilities. Especially, two strains of *Str. faecalis* produced more than 30 mg% of aromatic compounds after incubation at 37°C for 5 days. The isolated strains of *Leuconostoc* and *Lactobacillus* genera had lower aroma-producing abilities than streptococci.

IX. Proteolytic action of lactic acid bacteria isolated from raw milk. Rinjiro SASAKI and Toshitaka NAKAE (Jap. Journ. Zootech. Sci. **30**, 204–206, 1959)

Studies were made on the proteolytic action of 258 strains of lactic acid and 12 strains of other families of bacteria which had been isolated from raw milk in Japan in the previous studies. The proteolytic activity was estimated by colorimetric determination of free tyrosine liberated from proteins by incubation of the bacterial strains in skim milk.

The maximum value of free tyrosine content increased by protein decomposition was 4.38 mg% for *Streptococcus faecalis* A-13 and 4.32 mg% for *Str. lactis* B-5. Many of the strains having remarkable proteolytic action were found among the isolates of *Str. lactis*, *Str. cremoris*, and *Str. faecalis*, but lactobacilli showed generally weaker proteolysis than streptococci. Of the 12 strains of bacteria other than lactic acid bacteria, several were found to have strong proteolytic action.

Several strains of *Str. faecalis* and *Str. lactis*, which have both mid acid-producing capacity and strong proteolytic activity, may be utilized as cheese starter.

X. Growth stimulants of lactic acid bacteria. Rinjiro SASAKI, Tomokichi TSUGO and Toshitaka NAKAE (Jap. Journ. Zootech. Sci. **30**, 207–211, 1959)

Influences of removal of calcium ion and addition of plant and animal components on the growth of lactic acid bacteria were studied.

Removal of calcium ion from a modified milk by addition of 0.1–0.3% hexametaphosphate stimulated the acid production of lactic acid bacteria, especially *Lactobacillus acidophilus* and *L. bifidus*.

The acid production of lactic acid bacteria was stimulated remarkably by liver extracts, corn steep liquor, chondroitin sulfuric acid, and chitin hydrolyzates when they were added to skim milk or other media, but not by such natural components as peptone, yeast extracts and tomato juice.

Above all, chitin hydrolyzates treated with sulfuric acid were best as sources of bifidus factor.

73. General properties of animal fibers. III. Reactivity of keratin for caustic alkaline agent. Makoto KAMIYA (Jap. Journ. Zootech. Sci. **30**, 300–304, 1959)

Load-extension curves of wool and hair were determined before and after treatment with sodium hydroxide. The following results were obtained.

1) Wool and hair showed fundamentally common figures of load-extension curve.

2) Differences between wool and hair were elucidated, in detail, in relation to the fineness, tensile strength, and extension ratio. They were considered to be due to the existence of medullary strings in hair.

3) A phenomenon so-called mercerization of wool was recognized in the determination of load-extension curve and the estimation of reduction in work. Such phenomenon of wool thread was presumed to have been caused not by chemical changes of a single wool fiber, but agglutination of its outer scales.

74. Comparison between low pressure polyethylene can and metal can as milk container. Tomokichi TSUGO, Susumu KOYAMA and Sadaji ITO (Journ. Jap. Soc. Food and Nutrition, **12**, 348-352, 1959)

From the standpoint of effect on keeping quality of milk, the comparison was made between low pressure polyethylene can and metal can as milk container.

Several trials of storage tests at room temperature (in summer) and low temperature (3°C) were carried out. Analyses were made on acidity, total bacterial count, vitamin C determination, organoleptic observation, and alcohol test. Samples were collected at intervals of 4 hours for 16 hours in room temperature and 24 hours for 120 hours in low temperature test, respectively.

Data obtained showed that there was no marked difference in effect on keeping quality of milk between polyethylene can and metal can except for some superiority of the former in bacterial count and flavor.

75. Phosphatase of fat-globule membrane of cow's milk. I. Characteristics of phosphatase in cream. Susumu KOYAMA (Jap. Journ. Zootechn. Sci. 31, 88–91, 1960)

Several experiments were carried out on the effects of dilution, dialysis, pH, proteolytic enzyme, and inorganic compounds upon phosphatase activity in the cream.

The results obtained show that dilution or dialysis decreased phosphatase activity. This may be due to the dilution or loss of some activators which coexisted in the cream during dilution or dialysis.

Phosphatase in the cream was stable at such pH value as in the range of between 6.6 and 10.0, but became rapid when the pH was below 5.0.

Addition of pancreatin to the cream seemed to produce some substances activating phosphatase during incubation at 37°C.

A slight degree of reactivation of enzyme was observed when the cream was heated at 65°C for 5 seconds and incubated at 4°C for 18 hours.

Phosphatase was activated by Mg^{++} (at 23 ppm), but inhibited by Zn^{++} (at 65 ppm).

II. Reactivation of membrane phosphatase. Susumu KOYAMA (Jap. Journ. Zootechn. Sci. 31, 92–95, 1960)

It was shown by the author in his recent studies that the fat-globule membrane of cow's milk carried two kinds of phosphatases. One of them was associated with fat-globule membrane lipoprotein (M-P), and the other was contained in the supernatant (M-S). These two phosphatases differed in solubility in 0.08 M NaCl solution and in electrophoretic property.

In the present work, a comparison was made between the two phosphatases with respect to the effects of divalent metals and heating up on enzyme activity and reactivation of enzyme.

The results obtained show that there was no marked difference in the above-mentioned respects between phosphatases M-P and M-S, except for some superiority of the former in reactivating tendency.

76. Enzymatic determination of glucose in cow's milk using glucose oxidase and variations

of cow's milk. Tomokichi TSUGO and Yoshikazu KOGA (Jap. Journ. Zootechn. Sci. 30, 358–362, 1960)

A method for quantitative determination of glucose in cow's milk using glucose oxidase was investigated and its condition was established. As the result of tests using this method on 3 ml samples of cow's milk to which 1, 2 or 3 mg of glucose were added the recovery rate was proved to be from 97 to 102 per cent.

Glucose contents, as measured in 428 samples collected from 30 Holstein cows ranged from 0 to 32.6 mg, averaging 13.5 mg, per 100 ml.

The relationship with lactation stage was as follows. The glucose content of cow's milk was the highest between 45 and 105 days after calving and then decreased gradually to the end of lactation. In cow's yielding 16 to 25 l of milk per day, the milk contained more than 14 mg of glucose per 100 ml. The content of glucose in milk of the first lactation was higher than that in milk of any later lactation.

In colostrum, the content was low, being less than 5 mg per 100 ml to the fifth day after calving, and approached to the value of normal milk, i.e., more than 10 mg, during 15 days after calving.

77. **On the fat-globule membrane materials of cow's milk. Part III. The properties of lipoproteins in cow's milk and their relations to phosphatase and xanthine oxidase.** Rinjiro SASAKI and Susumu KOYAMA (Journ. Agr. Chem. Soc. Jap. 33, 631–635, 1959)

It is generally recognized that the fat-globule membrane consists chiefly of a lipoprotein complex. It was also shown by recent studies that alkaline phosphatase is associated with lipoprotein particles in milk.

At the present, however, many questions concerning the lipoproteins of milk remain unsolved.

In the work described in this paper lipoproteins were isolated from cream using such techniques as salting-out and ultracentrifugation, and their electrophoretic and enzymatic properties were studied.

It was found that milk contained two or three lipoproteins. From results obtained by the paper electrophoretic technique, one of these lipoproteins showed a similar mobility to that of one component of casein, while the other two lipoproteins hardly migrated on paper electrophoresis.

Each of two or three lipoproteins had both alkaline phosphatase and xanthine oxidase activities.

Part IV. The fat-globule membrane lipoprotein and its enzymatic properties. Susumu KOYAMA (Journ. Agr. Chem. Soc. Jap. 33, 635–638, 1959)

Previous paper showed that milk contained two or three lipoproteins, and these lipoprotein fractions had both alkaline phosphatase and xanthine oxidase activities.

In this paper the fat globule membrane lipoprotein was isolated by ultracentrifugation of mixture of butter milk and butter serum separated from the washed cream.

As expected from the results reported previously, the fat globule membrane lipoprotien had the alkaline phosphatase activity. But the greater part of original enzyme activity of non-washed cream was removed into washed water during the washing process. The enzyme activity of four times washed cream was no more than 13% of that of non-washed cream.

Of the xanthine oxidase, contrary to expectation, authors could not detect the enzyme activity in the washed cream.

The fat globule membrane lipoprotein hardly migrated on the paper-electrophoresis.

After treatment of the fat globule membrane lipoprotein with alcohol and ether, about 80% of the alkaline phosphatase activity remained in lipid-free proteinous residue. The alkaline phosphatase activity of this residue was also activated by lactose as shown in the previous paper.

Part V. Interaction between milk proteins and lecithin in emulsification. Susumu KOYAMA (Journ. Agr. Chem. Soc. Jap. 34, 510–513, 1960)

With a view to studying contribution of milk proteins and lecithin to emulsification of milk, emulsifying power of sodium caseinate, lactalbumin and lactoglobulin in butterfat-phosphate-buffer emulsion was measured under such conditions as (1) only each of these proteins was added, (2) each protein mixed with lecithin was added (3) each protein was added after lecithin was dissolved in butterfat previously.

Results obtained were as follows; Emulsifying power of sodium caseinate was same in (1), (2) and (3), that of lactoglobulin was the highest in (3) and the lowest in (1), and that of lactalbumin was the highest in (1) and the lowest in (2). On the other hand, lecithin scarcely showed emulsifying power.

From these results, it was supposed that contribution of lecithin to emulsification of milk was mainly in making more close adsorption of lactoglobulin on the surface of fat globule.

Part VI. The properties of membrane phophatase. Susumu KOYAMA (Journ. Agr. Chem. Soc. Jap. 34, 514–517, 1960)

It was previously reported that both fractions (supernatant and precipitate) prepared from the fat-globule membrane meterials by means of ultracentrifugation had an alkaline phosphatase activity. In this paper, further investigation of phosphatases contained in both fractions was made in respect of effect of pH on enzyme activity, electrophoretic properties and solubility.

The method of preparation of the fat-globule membrane materials was similar to the technique reported previously. Ultracentrifugation was carried out by means of the Spinco L type apparatus.

It was found that there was scarcely difference in the effect of pH on activity between supernatant and precipitate.

After treatment of both fractions with ethanol-ether mixture, the lipid-free proteinous residues were submitted to solubility test and paper electrophoresis. Results obtained showed that the phosphatase contained in supernatant was mostly soluble in 0.08 M NaCl solution but the phosphatase contained in precipitate was mostly insoluble. Futhermore, the former easily migrated on paper electrophoresis, whereas the latter did not migrate.

78. **Heterogeneity of α-casein and rennin coagulation. Part III.** Kunio YAMAGUCHI and Tomokichi TSUGO (Journ. Agr. Chem. Soc. Jap. 33, 797–800, 1959)

Influence of rennin on interaction between two fractions of Warner's α-casein, that is calcium insoluble fraction (α_R-fraction) and calcium soluble fraction (crude κ-fraction), was studied. It was shown that α_R-casein and para-κ-casein (which was transformed from κ-casein by rennin specifically) formed complex irreversibly by warming the mixture to 35°C. Evidences are as follows: (1) Disappearance of colloidal aggregates in para-κ-fraction was observed after mixing it with α_R-fraction ($\alpha_R : \kappa=4:1$) and warming to 35°C by means of ultracentrifugal analysis (in phosphate buffer, pH 7.5, $\mu=0.2$). (2) Rapid disappearance of

opalescence was confirmed by warming the mixture from 0° to 35°C by measurement of transmission (in 0.085 M NaCl solution, pH 6.6). (3) Two peaks in electrophoretic pattern of the mixture, which corresponded to α_R-and para-κ-caseins, were found to associate into one peak by warming previous to electrophoresis (in phosphate-NaCl buffer, pH 7.3, μ=0.1). It was concluded that interaction between both fractions in unseparated α-casein was retained after rennin action.

79. **Influence of rennin on the interaction between α- and β-caseins.** Tomokichi TSUGO and Kunio YAMAUCHI (Journ. Agr. Chem. Soc. Jap. 33, 801–806, 1959)

Electrophoretic comparison of casein and paracasein was performed under various conditions in order to ascertain whether rennin affects on interaction between α- and β-caseins. The results are as follows.

(1) Relative area of apparent β-casein peak both in ascending and descending patterns decreases with increasing protein concentration from about 0.3 to 3% (both in veronal and phosphate buffers of pH 7.3 and μ=0.1). There is no difference between casein and paracasein in this tendency. Splitting in ascending α-peak of paracasein is more distinct in higher protein concentration or lower ionic strength.

(2) Marked interaction is observed with arising electrophoresis temperature above about 20°C. At 30°C, α- and β-peaks associate into one unhomogeneous peak. If pH is high (pH 11.1), however, α- and β-peaks are separated at such a high temperature. In these characteristics no difference is found between casein and paracasein.

(3) Abnormal and asymmetric pattern in calcium-containing buffer (veronal buffer containing about 0.005 M calcium chloride, pH 7.2, temperature 4°C) will probably also ascribe to interaction between α- and β-caseins. In this system the pattern of paracasein is considerably different from that of casein.

It is assumed that rennin does not split the α-β casein complex into each component and that casein in milk is clotted as such a complex by rennin at usual condition.

80. **On the development of turbidity of calcium-soluble fraction of α- casein by rennin.** Kunio YAMAUCHI and Tomokichi TSUGO (Journ. Agr. Chem. Soc. Jap. 33, 957–961, 1959)

Development of turbidity of calcium-soluble fraction of Warner's casein (κ-casein fraction) by rennin was studied by measuring transmission without no filter at various conditions. The fraction used was 0.4% solution of pH 6.6 and containing 0.085 M NaCl. The transmission curve consisted of three phases; the initial phase in which no marked turbidity was developed, the second phase in which turbidity increased rapidly and the final phase in which optical density reached almost maximum. The logarithm of the difference between maximum value of optical density (D_∞) and each value (D_t) at t minutes after rennin addition was rectilinearly related with time (t) except for the initial phase; $\log 100\,(D_\infty - D_t)/D_\infty = -K(t-t_i)+2$ (K, t_i: constant). The measure of the slope, that is K, was proportionally increased with increment of added rennin concentration within certain limit of rennin concentration. Temperature coefficient (Q) for the reaction was calculated from K values at different temperatures (15 41°C). The mean of $Q_{1°C}$ was about 1.08, which was the same extent as that of general enzyme reaction and not so high as that of protein denaturation. The change of calcium-soluble fraction was assumed to consist of two processes overlapped each other, that is enzymic transformation of κ-casein into para-κ-casein and the aggregation process of the latter. As D_∞ was increased with temperature, the latter process itself was also considered to be dependent on temperature. Horse serum was found to inhibit the development of turbidity. The

inhibition was greater when the serum was added before rennin addition than when added at the beginning of the second phase.

81. **Comparison of α-casein and para-α-casein. Part. I.** Kunio YAMAUCHI (Journ. Agr. Chem. Soc. Jap. 33, 1134–1138, 1959)

α-casein and para-α-casein were compared with respect to the coagulability by alkaline earth metal salts and by acid. Comparisons were also carried out on acid-base titration curve and calcium-binding property. The results were as follows.

(1) Solubility curve of para-α-casein changed from a gentle slope to a steep one against the abscissa of calcium chloride concentration with progress of rennin action. After completeness of the reaction para-α-casein precipitated at a critical calcium chloride concentration sharply. The critical concentration of various alkaline earth metal salts to coagulate para-α-casein increased in order of $BaCl_2 < CaCl_2 < SrCl_2 < MgCl_2$. Above about these critical concentrations α-casein became the state of opalescence.

(2) Acid coagulation region of para-α-casein was considerably wider than that of α-casein especially on the alkaline side of isoelectric point (acetate buffer; ionic strength 0.1). Solubility in urea solution of acid-coagulated para-α-casein was slightly lower than that of α-casein between 3 to 6 M urea concentration in the presence of 0.1 M sodium chloride.

(3) No significant difference of acid-base titration curve was detected except for the bent region between pH 9.2–10.2, where para-α-casein had slightly higher alkali-binding capacity.

(4) Comparison of calcium-binding capacity was carried out on unfractionated whole casein and paracasein by membrane equilibrium method. The difference between casein and paracasein seemed to be within experimental error.

Author concluded that coagulation of α-casein by rennin might not be attributed to the change of net charge or the calcium binding capacity as the whole of α-casein. Factors related should be rather searched in the special complexed composition and structure of α-casein.

Comparison of α-casein and para-α-casein. Part II. Kunio YAMAUCHI (Journ. Arg. Chem. Soc. Jap. 33, 1139–1144, 1959)

α-casein and para-α-casein (rennin-treated) were compared in several properties.

(1) There was no difference in titratable value of SH-groups and color intensity of diazo reaction, phenol reaction and biuret reaction. Difference was also undetectable as to wave length of maximum absorption in ultraviolet region at pH 6.4, 9.4 and 11.7. At pH 6.4 and 9.4 extinction of para-α-casein was somewhat higher than that of α-casein over the whole range measured. This difference, however, seemed to be attributed to the difference of turbidity. At pH 11.7 both spectra were completely identical.

(2) Difference of ultracentrifugal patterns was not detected at pH 11.7, where both α- and para-α-caseins dissociated into the state of monomer having a very symmetrical single peak of the almost same S value. If the samples were neutrized to pH 7.0 (in the state of polymer), however, the pattern of para-α-casein became more heterogeneous than that of α-casein.

(3) No difference was found in the rate of dephosphorylation of α- and para-α-caseins by prostate phosphatase. It seemed to be unlikely that change of phosphorus linkage had any essential bearing on the formation of para-α-casein.

82. **Specific liberation of non protein nitrogen from κ-casein fraction by rennin.** Tomokichi

TSUGO and Kunio YAMAUCHI (Bull. Agr. Chem. Soc. Jap. 24, 96–100, 1960)

κ-casein and crude λ-casein were separated from crude κ-casein (calcium soluble fraction of Warner's α-casein) by ultracentrifugation. Development of turbidity and liberation of non protein nitrogen (NPN) in crude κ-casein by rennin was assumed to be attributed to the change of κ-casein itself.

Liberation of NPN from α_R-casein (calcium insoluble fraction of Warner's α-casein) and purified κ-casein by pepsin and chymotrypsin was compared with that by rennin. It was shown that pepsin, as well as rennin, liberated a definite amount of NPN from κ-casein and scarcely liberated it from α_R-casein, while chymotrypsin liberated NPN from both fractions increasingly with the increment of reaction time.

83. **On the urea denaturation of paracasein.** Kunio YAMAUCHI and Tomokichi TSUGO (Bull. Agr. Chem. Soc. Jap. 24, 101–104, 1960)

Paracasein was found to differ from casein markedly in its behaviour to urea. The former is partially denatured by urea. Precipitate appears in paracasein which is dialyzed against calcium free buffer after treatment with 6.66 M urea at low temperature. The amount of precipitate is about 9% of total nitrogen when sodium paracaseinate is treated with 6.66 M urea at 2°C for 24 hours and dialyzed repeatedly against phosphate-NaCl buffer of pH 7.3 at 2°C. As the precipitate consists of small particles, shaking causes marked opalescence and it takes a considerably long time to fall off. It is assumed that this precipitate may have mainly originated from para-α-casein and possibly be related to para-κ-casein.

84. **Radioactive contamination of milk and milk products in Japan.** Rinjiro SASAKI, Tomokichi TSUGO, Susumu KOYAMA, Tatsuo SATO and Yoichiro OMOMO (Bull. Agr. Chem. Soc. Jap. 24, 105–111, 1960)

Radioactive contamination of milk and milk products in Japan was studied extensively. Samples were collected monthly or every season from several districts of Japan.

In this report, total radioactivity of raw milk and skim milk powder and strontium-90 (Sr-90) concentration of skim milk powder and sweetened condensed milk were measured. These experiments show that there is neither seasonal variation nor regional variation in total radioactivity, and that in the measurements of Sr-90 concentration the values in northern districts are higher than those in other parts of Japan.

85. **Effect of urea on the casein in milk in process of rennin coagulation.** Kunio YAMAUCHI (Journ. Agr. Chem. Soc. Jap. 34, 145–150, 1960)

Experiments were performed on the effect of adding urea on skim milk (A), skim milk treated with rennin but not coagulated yet (B) and skim milk coagulated with rennin (C). Into 10 ml. of skim milk or curd was added an adequate amount of concentrated urea solution after a given time from addition of rennin and the volume was adjusted to 20 ml. with water. The amount of precipitated protein was determined after centrifugation. C was dissolved above about 6 M urea concentration and transparent solution was resulted. A became transparent with increase of urea concentration. No difference between A and C dissolved in concentrated urea solution was found as to pH and viscosity.

Effect of urea on B was different from that on A and considerably complicated. When relatively low concentration of urea (3.3 M) was added into B, coagulation appeared rapidly. The amount of the coagulum formed there increased and the time required for the coagulation after addition of urea decreased with the progress of rennin action respectively. This suggests that intra-micelle reaction of casein in milk proceeds progressively by rennin

before curdling occurs. It seems that casein particle in B, which is yet stable, is once dissociated by urea and then the other fashion of arrangement of the casein which has become reactive by rennin occurs. This coagulum was redissolved at higher urea concentration. α- to β-casein ratio of the coagulum was almost the same as that of ordinary unfractionated casein.

86. **The change of casein in milk at different stages of coagulation by rennin.** Kunio YAMAUCHI (Journ. Agr. Chem. Soc. Jap. 34, 240-245, 1960)

Normal skim milk (SM) or skim milk containing added calcium chloride (SMca; SM 50 ml +2 M $CaCl_2$ 3 ml) were ultracentrifuged (78,000 G, 60 min., at low temperature) at different stages of rennin coagulation; (1) without rennin, (2) immediately after addition of rennin at 2°C, (3) after standing overnight in the presence of rennin at 2°C and (4) after coagulation occurred by treatment with rennin at 35°C. Rennin activity, nitrogen (total, non-casein, non-protein nitrogen), total calcium and total phosphorus in the supernatant were analyzed. It was estimated that about one half of added rennin was removed by centrifugation probably as a result of combination with casein particles. The remaining activity in the supernatant was essentially the same for the stages of 2–4.

About 10% of total casein was remained in the supernatant of SM even after primary reaction had been complete. This unprecipitable casein was absent in SMca except for the sample of stage 1, in which about 2% was remained. About 1% of non-protein nitrogen and about 4% of non-casein nitrogen were liberated by rennin action. No significant difference was found between stages 1–4 with regard to calcium / nitrogen and phosphorus/ nitrogen ratios of precipitate. It was also confirmed that hardening and shrinkage of curd were non-enzymatic reaction unrelated to continuing action of rennin, by experiment using horse blood serum as inhibitor of rennin.

87. **Studies on the effects of some physical conditions on the submerged mold culture. Part 2. On the two types of pellet formation in the shaking culture.** Joji TAKAHASHI and Koichi YAMADA (Journ. Agr. Chem. Soc. Jap. 33, 707–710, 1959).

The globose mold colonies (so called pellets) formed in the shaking culture were found to be classified into two groups, "flocculent type pellet" and "non-flocculent type pellet". In the forming process of flocculent type pellet, remarkable flocculences of germinating conidia were observed. Therefore, one pellet was formed by many hyphae germinated from different conidia. This was supported by the fact that the numbers of inoculated conidia divided by the numbers of formed pellets (S/P) were in the order of 10^2, and increased in proportion to the increase of inoculum size.

On the other hand, neither flocculences of conidia nor hyphae were found in the forming process of non-flocculent type pellet. Therefore, one pellet was formed by branching and globose growth of a hypha germinated from one conidium. This was supported by the result that the values of S/P were nearly 1. Whether the flocculences of conidia occur or not, depends on many culture conditions and characteristics of conidia of each mold strain. For example, typical strains that belong to the flocculent type, are *Asp. niger* NRRL–337 and *Asp. oryzae* 0–3–6. On the other hand, *Pen. chrysogenum* Q–176 and *Asp. saitoi* BL–18–23 belong to non-flocculent type.

Part 3. Relations between the mycelial form of mold and the viscosity of mycelial suspension. Joji TAKAHASHI and Koichi YAMADA (Journ. Agr. Chem. Soc. Jap. 34, 100–103, 1960)

Rheological studies on the stable mycelial suspensions, which were prepared by suspending different forms of mycelia of *Asp. niger* NRRL-337 and *Pen. chrysogenum* Q-176 in the 15% soluble starch solutions, were carried out.

As shown in the following table, proportional relationships were obtained between mycelial forms and viscosities of mycelial suspensions. That is, the viscosity of the mycelial suspension increases according as the mycelial form of suspended mold becomes more filamentous and more homogeneous.

These results show that the viscosity of mycelial suspension is very available to express the complex states of mycelia, including not only pellet and pulp but also the intermediate forms.

Mycelial form	Viscosity of suspension*1 (readings of viscometer)*2
Large compact pellet (diameter 2-3 mm)	about 20
Small coarse pellet sprinkling of filamentous mycelia	40-90
Filamentous but heterogeneous form sprinkling of small coarse pellets	100-200
Filamentous and homogeneous form	200-400

*1: 0.5 g of dry mycelium/100 ml. of 15% soluble starch soln.

*2: Rotary drum type viscometer (rotor speed 60 r.p.m.) was used.

Part 4. Effects of the culture fluid viscosity on the mycelial forms of molds. Joji TAKAHASHI, Yoshiyasu KOSANO and Koichi YAMADA (Journ. Agr. Chem. Soc. Jap. 34, 440-442, 1960)

In the shaking culture of mold, it is known that the mycelium may grow into the homogeneous and filamentous suspension (pulp type growth), or develop into the form of globose colony (pellet type growth).

Among many physical conditions, which were considered to affect the type of mycelial growth in culture, the effects of viscosity of medium on the mycelial forms of *Asp. niger* NRRL-337 and *Pen. chrysogenum* Q-176, were investigated respectively in this paper.

In the case of *Asp. niger*, the flocculences of germinating conidia were observed in the culture fluid of lower viscosity (1-5 c.p.), however, this phenomenon could not be found in that of higher viscosity (more than 20 c.p.).

Therefore, the pellet type growth which originated from the flocculences of inoculated conidia, was prevented by the addition of suitable viscous materials (such as dextran and Na-alginate) to the culture fluid, and the more viscous the medium became, the more homogeneous and more filamentous mycelial growth was observed.

In the culture of *Pen. chrysogenum* in which the flocculences of conidia could not be found in the culture fluid of lower viscosity, little effect of medium viscosity on the mycelial forms was observed.

Part 5. Effects of the culture fluid viscosity on the growth and amylase production of *Asp. niger* NRRL-337. Joji TAKAHASHI, Haruo MACHIDA and Koichi YAMADA (Journ. Agr. Chem. Soc. Jap. 34, 442-446, 1960).

As reported in the previous paper, the mycelial form of *Asp. niger* NRRL-337 is remarkably affected by the culture fluid viscosity, that is, the more viscous the medium becomes, the more filamentous and more homogeneous form of mycelium is obtained. Therefore, it is expected that the mycelial growth and amylase production, in the shaking culture of this mold, may be influenced by the viscosity of medium. From this point of view, some experiments on amylase formation by *Asp. niger* NRRL-337 are carried out concerning with the viscosity of medium, and following results are obtained.

(1) When the mycelium grew into the pellet form in the culture fluid of lower viscosity (1-5 c.p.), the yield of mycelium per unit sugar consumed was very high (70-100%) in the early stage of growth, but it decreased to 10-20% in the later stage of growth. On the contrary, when the mycelium grew into the pulpy state in the medium of higher viscosity (20-50 c.p.), the yield of mycelium per unit sugar was nearly constant (40-60%) throughout the whole stages of growth. As the result, maximum yield of mycelium was obtained at the viscosity of 3-5 c.p.

(2) As shown in the following table, the yields of α-amylase per unit weight of mycelium (A/W) were remarkably affected by the viscosity of the culture fluid. The value of A/W increased in proportion to the increase of the medium viscosity, that is, the more filamentous and more homogeneous the mycelium became, the better production of α-amylase was obtained.

Viscosity of culture fluid c.p.	Max. yield of dry mycelium (W) g/100 ml.	Max. yield of α-amylase (A) unit/ml.	A/W
1	1.15	18.2	15.8
4	1.40	22.2	15.9
20	1.20	22.2	18.5
50	0.93	22.2	24.1

88. **Studies on L-glutamic acid fermentation. Part I. Isolation of a L-glutamic acid producing strain and its taxonomical studies.** Yuan-Chi Su and Koichi Yamada (Amino Acids, 1, 38-44, 1959 and Bull. Agr. Chem. Soc. Jap. 24, 69-74, 1960)

In recent years, a new practical method for the preparation of L-glutamic acid has been devised. This method is concerned with the incubation of certain microorganisms in the medium containing glucose, ammonium salt and minerals.

The authors have attempted to screen the new microorganisms which would produce L-glutamic acid directly from glucose resulting in the discovery of *Brevibacterium divaricatum* nov. sp., which gave a higher yield. This paper is concerned with the distribution of L-glutamic acid producing strains in nature and some taxonomical studies of this new strain.

The results obtained were as follows;

1. Five strains having exceedingly high L-glutamic acid productivity were obtained.
2. Most of these strains were found to belong to the Genus *Brevibacterium*. "*Brevibacterium divaricatum* nov. sp." was the name proposed by the authors for the new group of these strains. The properties of *Brevibacterium divaricatum* No. 1627 was as follows;

I) Microscopic observation: Cells are straight rods with rounded ends, 0.8 to 1.0, by 1.5 to 2.0 micron. Usually single, occasionally in pairs or irregular masses. Elongated forms of 2.0 by 6.0 microns are often seen. No capsules or endospores observed. Non-motile, no flagella and gram-positive.

II) Agar colonies: Abundant growth, punctiform, smooth, raised, glistening, yellowish-gray, non-viscid. Edge is entire.

III) Agar slant: Moderate growth, pale yellowish-gray, beaded to filiform, glistening, non-viscid. Medium unchanged.

IV) Gelatin stab: None or scanty liquefaction.

V) Nutrient broth: Slightly turbid, yellowish-brown flocculent sediment, no odor. Amount of sediment is abundant.

VI) Physiological properties:

1. Temperature relations: Optimum temperature 28–30°C, Maximum temperature 37°C, Minimum temperature 10°C, Thermal death point at 62°C for 10 minutes.
2. Litmus milk: Slightly acid production. Soft coagulum is formed. No peptonization on the 3rd day. Reduction of litmus begins on the 4th day, ends on the 14th day.
3. Indole is not produced.
4. Nitrates not reduced to nitrites.
5. Ammonia is not produced.
6. Hydrogen sulfide is not produced.
7. Methyl red test, negative.
8. Voges-Proskauer test, negative.
9. Catalase, strongly positive.
10. Urease positive.
11. Reaction to free oxygen: Aerobic.
12. pH range: Optimum pH 7.0 to 8.0.
13. Biologic relationships: saprophytic.
14. L-glutamic acid is accumulated in a large quantity, aerobically in the presence of carbohydrates, ammonium ion and inorganic salts.
15. Acid formation from carbohydrates: Acid is produced from glucose, fructose, mannose, sucrose, maltose and glycerol. In all cases gas formation is not recognized.
16. Source: Isolated from uncultivated soil.

Part II. Fermentative production of L-glutamic acid from glucose by *Brevibacterium divaricatum* nov. sp. Yuan-Chi Su and Koichi Yamada (Amino Acids, 1, 38–44, 1959 and Bull. Agr. Chem. Soc. Jap. 24, 140–146, 1960)

In a previous paper, both morphological and physiological properties of a new group of strains, including strain No. 1627, which as the only producing L-glutamic acid was isolated in a good quantity, have been examined. The name, *Brevibacterium divaricatum* nov. sp. was proposed to this strain because it was believed to be fallen under the head of a new species of genus *Brevibacterium* after it was contrasted with Bergey's Manual, 7th Ed.

This paper deals with the accumulation of L-glutamic acid from glucose by *Brevibacterium divaricatum* nov. sp.. A detailed study of culture conditions favoring the accumulation of L-glutamic acid was reported in this paper, and a discussion concerning the mechanism of L-glutamic acid formation was also presented.

These results can be summarized as follows;

(1) With regard to the constituents of the fermentation medium, various nitrogen sources, glucose and their optimum concentrations, as well as the additive influences of various growth substances and metals, were examined. Solely considering its worth in industrial application, urea seems to be a satisfactory nitrogen source. Its optimum concent-

ration in the medium containing 5% of glucose was found to lie between 0.67 to 0.9%. The growth of cells should be suppressed when the initial concentration of glucose exceeds 12.5%; therefore a level of 10% gives a better result. Data showed that addition of meat and bran extracts gave an increment of about 26% in acid yield.

(2) As for the fermentation condition, after experiments on the incubation temperature, pH and the influence of oxygen supply, etc., reveal that the aerobic condition is favorable for cell growth and accumulation of L-glutamic acid. An oxygen supply around 5×10^{-6} of K_d is desirable. The optimum incubation temperature was found to lie at 30°C.

A reasonable quantity of L-glutamic acid will be produced if the culture is kept neutral or preferably slightly alkaline by feeding a proper amount of urea aqueous solution throughout incubation.

(3) As a conclusion of the above experimental results, the yield of L-glutamic acid reached to 45% on the basis of glucose supplied after 30 hours' incubation.

(4) By-products such as alanine, α-ketoglutaric acid, pyruvic acid and succinic acid were found at the same time in the fermentation of L-glutamic acid employing *Brevibacterium divaricatum* nov. sp..

By observing the time of formation, the kind and the amount of these by-products it was presumed that the L-glutamic acid formation with this strain most probably proceeds from glucose to pyruvic acid, and then to α-ketoglutaric acid via Krebs TCA cycle. L-glutamic acid could be considered to be formed from α-ketoglutaric acid. The reductive amination reaction of α-ketoglutaric acid is assumed to be the main pathway of L-glutamic acid synthesis.

Part III. Pilot plant scale test of the fermentative production of L-glutamic acid by *Brevibacterium divaricatum* nov. sp. Yuan-Chi SU and Koichi YAMADA (Amino Acids, 2, 72–76, 1960 and Bull. Agr. Chem. Soc. Jap. 24, 525–529, 1960)

In this paper emphasis is placed on the utilization of fermentative production of L-glutamic acid in the pilot plant with formerly isolated *Brevibacterium divaricatum* nov. sp. using 500-L and 50-KL fermenters.

It was found that the yield of L-glutamic acid was higher than 45% in each case on the basis of consumed glucose when the hydrolysates of Cassava starch (Tapioka flour) was employed as sugar source.

The greatest advantage observed in this experiment was the remarkable reduction in time required for fermentation in comparison with the fermentation time necessary for the laboratory method. This must be a very favourable factor for the industrial preparation of L-glutamic acid.

89. Fundamental studies on the aerobic fermentation. Part VII. Oxygen transfer in gluconic acid fermentation by *Asp. niger*. Koichi YAMADA and Toshimasa YANO (Journ. Agr. Chem. Soc. Jap. 34, 523–527, 1960)

1. The effect of oxygen absorption rate of the medium on gluconic acid fermentation by *Asp. niger* van Tieghen No. 2 IAM 2093 was investigated. The maximum glucose uptake rates of the culture observed under different conditions were rather lower than those expected from the oxygen absorption rates of the medium.

From the facts that (I) the culture kept an ability to uptake glucose at a higher rate, (2) decrease of the oxygen absorption rate of the medium caused by growth of the mycelium could not explain such great difference, and (3) dissolved oxygen would be found in the culture medium even when the mycelium was uptaking glucose at the maximum rate, we

made a conclusion that the limiting factor of consumption of glucose was the intra-clump resistance of the mycelium against oxygen transfer.

2. The effect of mold pellet size, using *Asp. niger* NRRL 337, on QO_2 of the mycelium was investigated. QO_2 of mold pellets which were larger than 0.3 mm in diameter decreased in hyperbolic manner against increase of pellet size. According to the theoretical consideration based on the assumption of the homogeneous distribution of mycelium in a pellet, oxygen concentration in a culture medium should be increased in proportion to the square of the pellet diameter in order to maintain sufficient oxygen supply up to the interior mycelium of the pellet.

90. Studies on the utilization of hydrol. Part I. The production of d-saccharic acid by the nitric acid oxidation of hydrol. (1). Shigeo KIYOOKA (Journ. Agr. Chem. Soc. Jap. 34, 170–173, 1960)

Systematic experiments were carried out to find the best condition of the nitric acid oxidation of hydrol (the by-product in manufacturing dextrose) to d-saccharic acid under the following conditions. Nitric acid concentration: 30–62%, corresponding 3–6 moles per mole of dry substance of hydrol, reaction temperature: 55–93°C, the reaction time: 7 minutes-3 hours. On the results of the experiments, the best condition of the oxidation was found at higher temperature and lower acid concentration than in case of glucose, and the maximum yield of d-saccharic acid was 31.2% of the theoretical based on dry substance of hydrol.

The above hydrol contained 34.21% glucose, 15.38% disaccharides, 6.28% higher oligosaccharides, 49.17% moisture, and 0.06% ash.

Part II. The production of d-saccharic acid by the nitric acid oxidation of hydrol. (2) Shigeo KIYOOKA (Journ. Agr. Chem. Soc. Jap. 34, 173–177, 1960)

Hydrol was oxidized to d-saccharic acid (I) by nitric acid, using potassium nitrite or sodium meta-vanadate as catalysts.

1. In the case of potassium nitrite, the reactions were carried out under the following conditions. Nitric acid: 30–60%, corresponding 3–4 moles per mole of dry substance of hydrol, potassium nitrite: 1–7%, reaction temperature: 16–93°C, and the time: 2–88 hours.

By addition of nitrite, the yield of (I) did not increase, but did not decrease even in lowering the acid concentration or the reaction temperature. The maximum yield of (I) was 30.8% of the theoretical based on dry substance of hydrol.

2. In the case of vanadate, the reaction conditions were as follows.

Nitric acid concentration: 43–60%, corresponding 4–6 moles per mole of hydrol. Sodium meta-vanadate (tetra-hydrate) 0.001–0.1% of hydrol. Reaction temperature: 25–85°C, time: 0.7–88 hours.

Under a condition of 4 moles of nitric acid and 0.001% vanadate, the yield of (I) was 28–29%, but when the vanadate was added in the range of 0.005–0.01%, the yields decreased to 11–24%, and 10–17% dextro-tartaric acid (II), 7–26% oxalic acid (III), and a very small amount of racemic acid (IV) were found. If 0.1% of vanadate or 6 moles of nitric acid was used, (I) were 1–3%, (II) 3–5%, (III) 5–12% and (IV) 1–10%.

Part III. The production of calcium d-gluconate by electrolytic oxidation of hydrol. Shigeo KIYOOKA (Journ. Agr. Chem. Soc. Jap. 34, 262–267, 1960)

Electrolytic oxidation of the hydrol was carried out under the following conditions. Potential difference (direct current): 2–15 volts, current density: 1–5 amp./dm^2, quantity of electricity passed through: 100-125% of the theoretical, electrode: graphite plate. Elect-

rolyte; concentration of hydrol: 21-38%, buffer: calcium carbonate, and reaction temperature: 25-30°C.

When the amount of reducing sugar in the hydrol was assumed as that of glucose in order to calculate the apparent theoretical quantity (I) of electricity required for complete oxidation of hydrol and (I) was passed through, 88.5-93.3% of initial reducing sugar was oxidized, and finally 89.3-96.3% of that was oxidized by passing through over 100% of (I).

Thus, the yield of calcium d-gluconate based on dry substance of hydrol was found to be 43.5-77.1%.

91. Enhancement of the over-all lethal effect of ionizing radiations on microorganisms by sodium chloride. Yoshishige OKAZAWA, Mitsuo NAMIKI, Satoru YAMASHITA and Akira MATSUYAMA (Bull. Agr. Chem. Soc. Jap. 24, 235-242, 1960)

Aiming at the enhancement of total lethal effect of radiations, chemical agent actions on irradiated cells were investigated. Survival curves of several strains of yeasts and bacteria were determined, using the ^{60}Co gamma ray source of 270 curies.

It was found that the sensitivity of irradiated cells to NaCl was markedly increased by radiation. Enhancement of radiation lethal effect on yeasts and bacteria by pre- and post-treatments with NaCl is illustrated in this paper. This effect was not found with bacterial spores. Enhancing effect of NaCl during irradiation was clearly observed with cells in phosphate buffer and the similar effect by the post-NaCl treatment with cells in nutrient media. Some microbiologidal aspects of radiation enhancement of NaCl sensitivity are described in connection with the possible mechanism. The fact that this phenomenon of radiation enhancement by post-NaCl treatment was observed in the case of the cells which gave the one-hit type survival curve suggests that radiation damage responsible for this phenomenon is not restricted to biological targets within cells.

III. Agricultural Economics

Abstracts Nos. 92—93

92. Measurement of the economic effects of land improvement. Ryōhei SHIMIZU (Journ. of Rural Economics, 30-4, 1-22, 1959)

1. Driven by urgent need for increased food production after the war, a large amount of public fund for social overhead capital was invested for land improvement. How to measure its economic effects has long been discussed. The author wants to propose here a method to measure the economic effects of land improvement from the national economic point of view.

As land improvement produces far-reaching effects extending over a long time, there is a need to consider not only of its direct effects, but also of its indirect effects. As a result of public investment for land improvement, artificial fertility is added to the original and natural fertility of the soil, and the land becomes more productive than before. The author thinks that the increase in differential rent caused by the investment is a proper measure of production effects of land improvement. He proposes, in this paper, the adoption of production function for its estimation.

Land improvement is the investment of labour and capital in land to increase its fertility. Therefore, the simplest way one may think of in estimating the effects of land

improvement is to compare outputs before and after the improvement, or to compare output of improved area with that of unimproved area of the similar nature. If the effects of land improvement consist of the increase in production alone (if there were no change in production cost), we can measure its effects by simply measuring the increase in physical output. However, since the increase in physical output is usually accompanied by change in production cost, we have to take into consideration the change in cost in order to make our estimate meaningful.

2. The "simplest" method above-mentioned is not quite so simple as may first be conceived. The method is to measure the difference in output of the same land before and after the improvement. But there are several other factors besides the land improvement which influence the increase in output. Firstly, yields of agricultural products are greatly influenced by weather conditions. In order to eliminate the influence of weather we have to use mean values based on several normal yields before and after the investment. Secondly, we have to consider the increase which may be caused by the progress in agricultural techniques—improvement in breeding, improvement in cultivation, and so forth. This "natural increase" must be eliminated. In order to measure the net effect of land improvement based on the assumption, other things remaining constant, we may first select an unimproved area which has soil conditions similar to the improved one, and we may next proceed to subtract the increase in output due to technological progress from the total increase in output. The way to measure the increase in the output due to technological progress is as follows: measure the difference in output of unimproved area between periods before and after the land improvement, and this may safely be regarded as the "natural increase" during the periods under consideration.

3. In the above-mentioned method, the change in physical output has only been considered. We did not take account of the change in farming practices due to land improvement, namely, the change in input or the change in production cost. If we want to make our estimate more meaningful, we shall have to take these factors into account which have rarely been done in "conventional" studies.

In addition to this limitation, the conventional method above-mentioned has another difficulty. To measure the change in physical output, time series data of output before and after land improvement are needed. However, it is not very easy to obtain them in practice. Or, if available, they tend to prove useless, because of the actual difficulties of ascertaining the level of yields relative to the normal yield, so to speak. We thus have to find some other ways.

The method the author is going to propose here is the one of measuring the economic effect of land improvement by means of production functions based on cross sectional data.

First, let us define the production function for the non-irrigated (unimproved) area.

$$P_\alpha = \alpha_0 + \alpha_1 L + \alpha_2 C_1 + \alpha_3 C_2 + \alpha_4 T \qquad (1)$$

where, P_α: output L: labour C_1: operating capital C_2: fixed capital

T: land $\alpha_0, \alpha_1, \alpha_2, \alpha_3, \alpha_4$: parameters

Next, let us define the production function for the irrigated area (improved area), which has similar qualities with the non-irrigated area except that farmers in the former can use public fixed capital ΔC_2, in addition to their private fixed capital C_2. That is, the total fixed capital used for production by farmers in the irrigated area is $C_2 + \Delta C_2$. The production function for the irrigated area may be written as follows,

$$P_\beta = \alpha_0 + \alpha_1 L + \alpha_2 C + \alpha_3 (C_2 + \Delta C_2) + \alpha_4 T . \qquad (2)$$

Let us assume that ΔC_2 consists of two parts,

$$\Delta C_2=\gamma_0+\gamma_1 T \qquad (3)$$

where,

γ_0: a part of the public fixed capital, the gain of which is not proportional to the acreage cultivated (T).

γ_1: a part of the fixed capital, the gain of which is proportional to T.

Substituting (3) into (2), we get

$$\begin{aligned} P_\beta &= \alpha_0+\alpha_1 L+\alpha_2 C_1+\alpha_3\{C_2+(\gamma_0+\gamma_1 T)\}+\alpha_4 T \\ &= (\alpha_0+\alpha_3\gamma_0)+\alpha_1 L+\alpha_2 C_1+\alpha_3 C_2+(\alpha_4+\alpha_3\gamma_1)T \\ &= \beta_0+\alpha_1 L+\alpha_2 C_1+\alpha_3 C_2+\alpha_5 T \qquad (4) \end{aligned}$$

where $\beta_0=\alpha_0+\alpha_3\gamma_0$, $\alpha_5=\alpha_4+\alpha_3\gamma_1$

Comparing (4) with (1), it may be clear that the constant term and the coefficient of land are different and other parameters completely the same. That is, by the land improvement investment the land in the irrigated area has acquired artificial fertility in addition to its natural fertility and thus becoming more productive than the non-irrigated area. To our eyes of production economist, productivity of labour and capital in the irrigated area has not changed from that in the non-irrigated area, with the level of production (the constant term) and the productivity of land alone changing from that in the non-irrigated area.

We can estimate the parameters of our production functions in both areas by using the data of individual farms in these areas. The production effects of land improvement can be expressed as follows,
from (1) and (4),

$$P_\beta-P_\alpha=(\beta_0-\alpha_0)+(\alpha_5-\alpha_4)T \qquad (5)$$

If in both areas the same amount of labour L, operating capital C_1, fixed capital C_2 and land T are used, output P_α will be produced in the non-irrigated area according to the production function (1), and the output P_β will be produced in the irrigated area according to the production function (4). Then, we may regard the difference, $P_\beta-P_\alpha$, as the production effect of public investment in irrigation.

In other words, the relationship (5) shows the production effect of the irrigation for the farm cultivating land T. Let us assume that data of output P, labour L, operating capital C_1, fixed capital C_2 and land T are available for m farms picked up at random in the non-irrigated area and the similar data are also available for n farms in the irrigated area. We can apply the least-squares to determine parameters, $\alpha_0, \alpha_1, \alpha_2, \alpha_3, \alpha_4, \alpha_5, \beta_0$, for production functions in both area. The least-squares method can be applied separately to equations (1) and (4).

4. However, it is our fundamental assumption that productivities of labour, operating capital and fixed capital, namely $\alpha_1, \alpha_2, \alpha_3$, are the same between the irrigated area and the non-irrigated area. If we apply the least-squares separately, then there is no guarantee that α_1, α_2, and α_3 will be estimated identically between two areas. In order to avoid this danger, we compile our data for areas, and apply the least-squares to these compiled data. By doing so additional advantage may be obtained, that is, the increase in the degree of freedom.

We introduce following notations for notational convenience. Let production function in the non-irrigated area be,

$$Y_\alpha=\alpha_0+\alpha_1 X_1+\alpha_2 X_2+\alpha_3 X_3+\alpha_4 X_4+S_\alpha \qquad (6)$$

And let production function in the irrigated area be,

$$Y_\beta=\beta_0+\alpha_1X_1+\alpha_2X_2+\alpha_3X_3+\alpha_5X_5+S_\beta \tag{7}$$

where

Y_α: output in the non-irrigated area
Y_β: output in the irrigated area
X_1: amount of labour
X_2: amount of operating capital
X_3: amount of fixed capital
X_4: land area in the non-irrigated area
X_5: land area in the irrigated area
S_α: stochastic random error of output in the non-irrigated area
S_β: stochastic random error of output in the irrigated area
α_0: constant term in the non-irrigated area
β_0: constant term in the irrigated area
α_1: marginal productivity of labour
α_2: marginal productivity of operating capital
α_3: marginal productivity of fixed capital
α_4: marginal productivity of land in the non-irrigated area
α_5: marginal productivity of land in the irrigated area

We now transform (6) and (7) into the following forms:

$$S_{\alpha i}=Y_{\alpha i}-(\alpha_0+\alpha_1X_{1i}+\alpha_2X_{2i}+\alpha_3X_{3i}+\alpha_4X_{4i})$$
$$i=1, 2, \cdots, m$$
$$S_{\beta j}=Y_{\beta j}-(\beta_0+\alpha_1X_{1j}+\alpha_2X_{2j}+\alpha_3X_{3j}+\alpha_5X_{5j})$$
$$j=1, 2, \cdots, n$$

Our problem is to determine the parameters, $\alpha_0, \beta_0, \alpha_1, \alpha_2, \alpha_3, \alpha_4, \alpha_5$.

$$D=\sum_{i=1}^{m}(S_{\alpha i})^2+\sum_{j=1}^{n}(S_{\beta j})^2$$

We minimize D with respect to α_0,

$$\frac{\partial}{\partial\alpha_0}\left\{\sum_{i=1}^{m}(S_{\alpha i})^2+\sum_{j=1}^{n}(S_{\beta j})^2\right\}=0$$

That turns out to be,

$$\sum_{i=1}^{m}S_{\alpha i}=0$$

$$\sum_{i=1}^{m}Y_{\alpha i}=m\alpha_0+\alpha_1\sum_{i=1}^{m}X_{1i}+\alpha_2\sum_{i=1}^{m}X_{2i}+\alpha_3\sum_{i=1}^{m}X_{3i}+\alpha_4\sum_{i=1}^{m}X_{4i}$$

$$\therefore\quad \alpha_0=\bar{Y}_\alpha-\alpha_1\bar{X}_1-\alpha_2\bar{X}_2-\alpha_3\bar{X}_3-\alpha_4\bar{X}_4 \tag{8}$$

where $\bar{Y}_\alpha=\frac{1}{m}\sum_{i=1}^{m}Y_{\alpha i}$, $\bar{X}_k=\frac{1}{m}\sum_{i=1}^{m}X_{ki}$

$(k=1, 2, 3, 4)$

Similarly, by minimizing D with respect to β_0, we obtain

$$\beta_0=\bar{Y}'_\beta-\alpha_1\bar{X}'_1-\alpha_2\bar{X}'_2-\alpha_3\bar{X}'_3-\alpha_5\bar{X}'_5 \tag{9}$$

where $\bar{Y}'_\beta=\frac{1}{n}\sum_{j=1}^{n}Y_{\beta j}$, $X'_l=\frac{1}{n}\sum_{j=1}^{n}X_{lj}$

$(l=1, 2, 3, 5)$

Next we minimize D with respect to α_1,

$$\frac{\partial}{\partial\alpha_1}\left\{\sum_{i=1}^{m}(S_{\alpha i})^2+\sum_{j=1}^{n}(S_{\beta j})^2\right\}=0 \qquad \text{i.e.} \quad \sum_{i=1}^{m}S_{\alpha i}X_{1i}+\sum_{j=1}^{n}S_{\beta j}X_{1j}=0$$

$$\text{i.e.} \quad \sum_{i=1}^{m}Y_{\alpha i}X_{1i}+\sum_{j=1}^{m}Y_{\beta j}X_{1j}=\alpha_0\sum_{i=1}^{m}X_{1i}+\alpha_1\sum_{i=1}^{m}X^2_{1i}+\alpha_2\sum_{i=1}^{m}X_{1i}X_{2i}+\alpha_3\sum_{i=1}^{m}X_{1i}X_{3i}$$

$$+\alpha_4\sum_{i=1}^{m}X_{1i}X_{4i}+\beta_0\sum_{j=1}^{n}X_{1j}+\alpha_1\sum_{j=1}^{n}X^2_{1j}+\alpha_2\sum_{j=1}^{n}X_{1j}X_{2j}+\alpha_3\sum_{j=1}^{n}X_{1j}X_{3j}$$

$$+\alpha_5\sum_{j=1}^{n}X_{1j}X_{5j} \tag{10}$$

Substituting from (8) and (9) into (10), we obtain

$$\alpha_1\left\{\sum_{i=1}^{m}(X_{1i}-\bar{X}_1)^2+\sum_{j=1}^{n}(X_{1j}-\bar{X}_1')^2\right\}+\alpha_2\left\{\sum_{i=1}^{m}(X_{1i}-\bar{X}_1)(X_{2i}-\bar{X}_2)+\sum_{j=1}^{n}(X_{1j}-\bar{X}'_1)(X_{2j}-\bar{X}'_2)\right\}$$

$$+\alpha_3\left\{\sum_{i=1}^{m}(X_{1i}-\bar{X}_1)(X_{3i}-\bar{X}_3)+\sum_{j=1}^{n}(X_{1j}-\bar{X}'_1)(X_{3j}-\bar{X}'_3)\right\}$$

$$+\alpha_4\left\{\sum_{i=1}^{m}(X_{1i}-X_1)(X_{4i}-\bar{X}_4)\right\}+\alpha_5\left\{\sum_{j=1}^{n}(X_{1j}-\bar{X}'_1)(X_{5j}-X'_5)\right\}$$

$$=\sum_{i=1}^{m}(Y_{\alpha i}-\bar{Y}_\alpha)(X_{1i}-\bar{X}_1)+\sum_{j=1}^{n}(Y_{\beta j}-\bar{Y}'_\beta)(X_{1j}-\bar{X}'_1) \tag{11}$$

Using following notations,

$$V_{kl}=\sum_{i=1}^{m}(X_{ki}-\bar{X}_k)(X_{li}-\bar{X}_l), \qquad V'_{kl}=\sum_{j=1}^{n}(X_{kj}-\bar{X}'_k)(X_{lj}-\bar{X}'_l)$$

$$V_{\alpha l}=\sum_{i=1}^{m}(Y_{\alpha i}-\bar{Y}_\alpha)(X_{li}-\bar{X}_l), \qquad V'_{\beta l}=\sum_{j=1}^{n}(Y_{\beta j}-\bar{Y}'_\beta)(X_{lj}-\bar{X}'_l),$$

the relationship of (11) is written as follows:

$$\alpha_1(V_{11}+V'_{11})+\alpha_2(V_{12}+V'_{12})+\alpha_3(V_{13}+V'_{13})+\alpha_4(V_{14})+\alpha_5(V'_{15})=V_{\alpha 1}+V'_{\beta 1} \tag{12}$$

In the same way by minimizing D with respect to α_2, α_3, α_4, and α_5 we obtain

$$\alpha_1(V_{21}+V'_{21})+\alpha_2(V_{22}+V'_{22})+\alpha_3(V_{23}+V'_{23})+\alpha_4(V_{24})+\alpha_5(V'_{25})=V_{\alpha 2}+V'_{\beta 2} \tag{13}$$

$$\alpha_1(V_{31}+V'_{31})+\alpha_2(V_{32}+V'_{32})+\alpha_3(V_{33}+V'_{33})+\alpha_4(V_{34})+\alpha_5(V'_{35})=V_{\alpha 3}+V'_{\beta 3} \tag{14}$$

$$\alpha_1(V_{41})+\alpha_2(V_{42})+\alpha_3(V_{43})+\alpha_4(V_{44})=V_{\alpha 4} \tag{15}$$

$$\alpha_1(V'_{51})+\alpha_2(V'_{52})+\alpha_3(V'_{53})+\alpha_5(V'_{55})=V'_{\beta 5} \tag{16}$$

From (12), (13), (14), (15) and (16), parameters $\alpha_1, \alpha_2, \alpha_3, \alpha_4,$ and α_5 are determined. Expressed in matrix form, the normal equations are

$$\begin{pmatrix} V_{11}+V'_{11} & V_{12}+V'_{12} & V_{13}+V'_{13} & V_{14} & V'_{15} \\ & V_{22}+V'_{22} & V_{23}+V'_{23} & V_{24} & V'_{25} \\ & & V_{33}+V'_{33} & V_{34} & V'_{35} \\ & & & V_{44} & 0 \\ & & & & V'_{55} \end{pmatrix}\begin{pmatrix}\alpha_1\\ \alpha_2\\ \alpha_3\\ \alpha_4\\ \alpha_5\end{pmatrix}=\begin{pmatrix}V_{\alpha 1}+V'_{\beta 1}\\ V_{\alpha 2}+V'_{\beta 2}\\ V_{\alpha 3}+V'_{\beta 3}\\ V_{\alpha 4}\\ V'_{\beta 5}\end{pmatrix}$$

Left half of the matrix is omitted since coefficients of the matrix are symmetrical.

5. By use of the above stated production function method, we are going to measure the effects of irrigation in the Asaka Canal. The canal irrigates about 6,000 chō of paddy field in Asaka District in Fukushima Prefecture. Data for our analysis are taken from "Reports on Production Cost of Rice" by the Statistics and Survey Division of Ministry

of Agriculture and Forestry.

Before proceeding to the application of the production function method, the economic effect of irrigation by the Asaka Canal is measured by comparing irrigation and non-irrigation areas simply in terms of average outputs and inputs. This parenthetical, so to speak, method is to clarify indirectly the superiority of the production function method over average output comparison method.

Using annual data on output P (koku), land T (tan), operating capital C_1 (thousand yen) and fixed capital C_2 (thousand yen) of sample farms, we can calculate mean value of output per tan P/T, labour per tan L/T, operating capital per tan C_1/T, and fixed capital per tan C_2/T.

These figures for the irrigation area and the non-irrigation area are shown in Table 1.

Table 1

Item \ Year / Area	1952 (good harvest) Irrigation area	1952 (good harvest) Non-irrigation area	1953 (bad harvest) Irrigation area	1953 (bad harvest) Non-irrigation area	1954 (bad harvest) Irrigation area	1954 (bad harvest) Non-irrigation area	1955 (good harvest) Irrigation area	1955 (good harvest) Non-irrigation area
P/T (koku per tan)	2.76	2.40	1.72	1.68	2.18	2.19	3.02	2.90
L/T (hour per tan)	216	248	216	230	170	211	191	214
C_1/T (thousand yen per tan)	6.8	5.8	6.3	6.3	5.7	4.7	5.9	4.9
C_2/T (thousand yen per tan)	9.9	6.0	12.0	12.0	8.9	8.9	8.6	9.5

It may easily be found from the comparison of outputs that the irrigation effect is evident only in 1952. In other years, there is no significant difference in the amount of production between irrigation and non-irrigation areas. We then proceed to the comparison of inputs per tan. In general, production is capital-intensive and labour-saving in nature in the irrigation area and is capital-saving and labour-intensive in the non-irrigation area. That is, capital is to be substituted for labour in the irrigation area. We cannot measure the production effects by simply comparing mean values of outputs of both areas. Here, the use of production function method is called for.

The results of measurement are as follows:

non-irrigation area — irrigation area

1955

$P_\alpha=-0.79+0.011L-1.74C_1+0.167C_2+3.49T$ $\quad P_\beta=1.87+0.011L-1.74C_1+0.167C_2+3.55T$

1954

$P_\alpha=-1.59+0.045L-0.622C_1+0.121C_2+1.695T$ $\quad P_\beta=0.49+0.045L-0.622C_1+0.121C_2+1.63T$

1953

$P_\alpha=-2.28+0.026L-0.339C_1-0.255C_2+1.81T$ $\quad P_\beta=4.94+0.026L-0.339C_1-0.255C_2+1.33T$

1952

$P_\alpha=1.05+0.017L+0.050C_1-0.017C_2+2.14T$ $\quad P_\beta=0.34+0.017L+0.050C_1-0.017C_2+2.74T$

Measuring units are:

Output P: koku Land T: tan Labour L: 10 hours

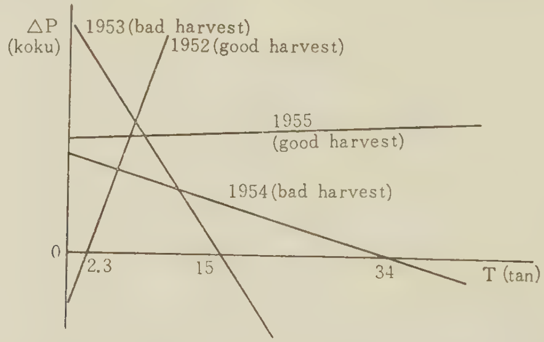
Operating capital C_1: 10,000 yen Fixed capital C_2: 10,000 yen

Economic interpretation of the results is as follows:

(i) The production effects of irrigation ΔP can be expressed as $\Delta P=P_\beta-P_\alpha$, the value of which is

1955	$\Delta P=2.66+0.06T$	
	$\Delta P>0$ for $T>0$	(17)
1954	$\Delta P=2.06-0.06T$	
	$\Delta P>0$ for $T<34$	(18)
1953	$\Delta P=7.22-0.48T$	
	$\Delta P>0$ for $T<15$	(19)
1952	$\Delta P=-1.39+0.60T$	
	$\Delta P>0$ for $T>2.3$	(20)

ΔP is shown as a linear function of T. The value of ΔP differs from year to year. The effects of irrigation are different in years of good, bad and normal harvest.



Based on the assumption of linear relation between ΔP and T over the range, the economic production effect of irrigation tends to be negative for larger scale farms in the years of bad harvest. This break-even-point of the production effect of irrigation is 1.5 chō in 1953 and 3.4 chō in 1954 respectively.

While larger scale farms gain greatly in the years of good harvest, especially 1952, the gain tends to be offset to the same extent by the loss in the years of bad harvest. This tendency is strengthened as farms become larger. Moreover, this diversion in the effect of irrigation between years increases the uncertainty of production in larger scale farms. It may be said that the farms under 1.5 chō can enjoy the gain due to irrigation irrespective of good or bad harvest. Certainly the results of the four years are too small a sample to make a definite statement. But still it appears that investment for irrigation should turn out to be of more help to small or middle scale farms. This may suggest that the public investment for land improvement is likely to work protectively particularly for

small and middle scale farms.

(ii) The value of irrigation canal as a capital asset can be calculated from the production effect of irrigation. The mean value of production effect is calculated as follows: from (17), (18), (19) and (20),

$$\Delta P_m=\frac{1}{4}\Big\{(2.66+0.06T)+(2.06-0.06T)+(7.22-0.48T)+(-1.39+0.60T)\Big\}=2.64+0.03T$$

Since average size of paddy field in sample farms in the irrigation area, T, is 13.9 tan, the mean value of production effect, ΔP_m, is 3.06 koku. That is, the production effects is 0.22 koku per tan. It may be said that farms in the irrigation area produce 0.22 koku more per tan than farms in the non-irrigation area. This difference in yield may well be assumed to be a result of the public investment for irrigation alone. Therefore, the total value of the irrigation canal as a rent yielding asset can be calculated as follows: 0.22 koku of rice valued at ¥10,000 per koku, or ¥2,200, be capitalized at seven per cent of interest rate. From this comes out the value of irrigation capital of ¥31,000 per tan. Since the irrigation area covers 60,000 tan, the total value of the irrigation canal amounts to ¥1,830,000,000.

Conversion Tables

Japanese unit	Metric unit	equivalents
1 tan	9.9174	ares
1 chō	0.99174	hectare
1 koku	1.8039	hectolitres
Weight of rice (Brown) per koku	150	kilogrammes

93. Land problems of the English Civil War, 1640–1660. Shigeaki SHIINA (Socio-Economic History Series, 4-1, 89-136, 1960)

I. Introduction The Englich Civil War in the seventeenth century has received so much attention from political and economic historians of many countries as well as English historians. Thousands of books and articles have been written on the problems of the Civil War, and in those works historians have tried to clarify the crucial points of the social and political antagonism during the Revolution. And yet, there remained some fields in which historical studies must be done more throughly and precisely. Particularly, little is known about the peasantry during that period. It was not until 1940's or 1950's that people began to be interested in the agrarian legislation of the English Revolution. Controversies, however, still continue only to increase the confusion which brings even such an idea as that English Revolution had no land problem which the Parliament had to solve during these two decades.

So far, the historical documents, especially the materials which would reveal the changes of the social and economic situations of the peasant class during and after the period, have scarcely been examined. This is the main reason why the land problems of the English Revolution are understood very little. A bourgeois revolution, if it deserves to be so called, must be thought to have land problems as it's issue of the first importance, and the land problems can not be clarified only with the examination of the revolutionary party's programs: Particularly, it is essentially important to take this point into consideration with regard to the English Revolution since the Parliamentarian agrarian legislation itself during that period did not include the abolition of the feudal landownership.

We must, therefore, proceed our studies in local or manorial history in connection with the agrarian legislation of the Puritan Revolution.

Firstly, it would be necessary to examine the change in the landownership before and

Table I

Owners of	1561	1601	1640	1680
1–4 manors	1445 (56.7)	1457 (57.2)	1638 (64.3)	1684 (66.1)
5–10 manors	490 (19.2)	544 (21.3)	488 (19.1)	556 (21.8)
10 manors or more	612 (24.0)	546 (21.4)	421 (16.5)	347 (13.6)
Total	2547(100.0)	2547(100.0)	2547(100.0)	2547(100.0)

after the Civil War, as our preliminary work. Table I, cited from Prof. Tawney's famous article "The Rise of the Gentry" shows the shift in the manorial property in the seven counties of Herts., Beds., Bucks., Surry, Worcs., Hants., and the North Riding of Yorks., at the four dates befere and after the Civil War. It is obvious from these figures that the ownership of 2547 manors in the seven counties changed remarkably during about one hundred and twenty years. The number of medium-sized properties, especially the number of manors belonged to owners with less than four manors increased throughout this period, while that of the largest, i.e. owners with ten manors and more, decreased significantly. This means, in another way, large properties belonged to the Crown and the nobles changed hands gradually, while medium-sized or small estates of the gentry were growing steadily in numbers, particularly during the four decades after the beginning of the Civil War. This tendency was undergoing notably from the time of the Dissolution of the Monasteries in 1530's, and was accelerated by the sales of Royalist lands during the Interregnum.

II. Land Transference during the Civil War It is well known that lands of crown, church and delinquents were sold by the Parliament during the Interregnum. Firstly, in 1643, their lands were sequestered by the Parliament to be managed by stewards or lessees whom the committee of the Parliament named. But this method was inefficient as the counter-measure to increase the Parliament's budget. In 1645, therefore, the Committee for Compounding was set up to carry out the sequestration of the Royalist property and assess the composition, or fine to be paid by those Royalists.

Royalists, otherwise called Delinquents, Papists, Malignants or Traitors, were compounded to the amount of about £1,305,000 during nine years from 1644 to 1652. The number of Royalists compounded were, for example, more than 800 persons in Lancashire, 677 in Yorkshire; and in two counties of Durham and Northumberland, they were no less than 150 only in two years i.e. 1649 and 1650. These delinquents whose lands were confiscated were mainly the men of property and belonged to the class of nobilities and the gentry class. Table II, which relates to Yorkshire and Durham shows the number of Delinquents according to their social status. In Yorkshire, total number of Delinquents whose lands were sequestered during nine years from 1645 to 1653, amounts to some 680; out of this total, 454 were all the men whose properties were estimated at £200 and more. It is obvious that the majority of this 454 consists of the nobles, knights, esquires and gentlemen. It is much the same in case of Durham and the other 226 persons of Yorkshire (Out of these 226 Delinquents whose property was under £200, at least 90 were the people who belonged to the class of the nobles and gentry. This is about 63% of those whose social status is known).

Viewed from another point, the same conclusion as mentioned above can be noticed; that is, as the following figures show, the nobility and the gentry paid by far the largest sum of compositions. Figures in Table III are based on S.I. Arkhangelsky's study ('Agrarian

Table II

	Yorks.	Durham
Nobles & Knights	44	21
Esquires	63	30
Gentlemen	199	67
Yeomen	45	32
Husbandmen	14	—
Merchants	28	—
Craftsmen	3	2
Officials	10	—
Clerics	8	2
Status unknown	40	20
Total	454	174*

* Number of Royalists whose land was sequestered in 1649 and 1650. Figures are all based on "Royalist Composition Papers".

Table III

	Number	Compositions
Nobles & Knights	89	£196,920
Esquires	206	179,902
Gentlemen	194	50,617
Yeomen	29	2,977
Husbandmen	2	30
Craftsmen	9	1,103
Merchants	26	8,707
Officials	7	8,583
Clerics	6	4,486
Lawyers	5	942
Professors	1	333
Status unknown	34	14,861
Total	608	469,461

legislation of the English Revolution, 1643–48'), and relates to the Delinquents who supported Lord Holland in Surry in 1648 together with the Royalists who took part in the Crown army at Oxford in the same year. As will be seen in this table, 489 out of the total 608 Royalists were the men belonged to the class of nobility and the gentry, and the sum paid by them amounts to some £427,440—this is about 90% of the total.

Meanwhile, it must be noticed in these two tables that there were also some people of the lower class such as yeomen, husbandmen and craftsmen. These people, however, had not always their own firm reason so that they had to be Royalists. Actually, most of them were relieved from paying the composition, swearing an oath that they would never commit again the error to support the Royalist.

In the third place, as for lands sequestered and sold by the Parliament, the greatest portion of sales were those of nobility and gentry. The first act for the sale of Delinquents' land was passed in 1651 and the task was carried out by the Drury House, another committee of the Parliament. According to the study of Arkhangelsky, social status of 781 delinquents whose lands were sequestered and sold is as follows: 34 nobilities, 284 gentry (including knights and baronets), 44 yeomen and husbandmen, 38 merchants and other professional men, and 381 people whose status unknown. Figures which relate to the sale in twelve counties are available and these are shown in Table IV.

In these twelve counties, the number of delinquents whose land was forfeited was fifty in all, and the number of the sales was 179. Of these 179 sales, about 90 per cent (156 in number) were those of nobilities and gentry. And that, as the table shows, 45 out of total 62 manors sold belonged to the nobles. In four counties of Berks., Oxon., Hants., and Sussex, 7 nobilities were forfeited of their 31 manors and other 12 estates, i.e. about 85

per cent of the total area of the lands sold. It may be necessary to add here that the number of estates other than manors belonged to the gentry is considerable—49 against 45 of the nobilities. But, generally speaking, those were rather small both in area and value.

Table IV

	Lands of nobility		Lands of gentry		Total
	Manors	Others*	Manors	Others*	
Beds.	3	—	3	2	8
Bucks.	4	—	1	1	6
Berks.	12	5	—	1	18
Hants.	10	4	1	11	26
Oxon.	4	—	3	—	7
Sussex	5	3	3	2	13
Essex	4	1	1	5	11
Herts.	2	—	1	2	5
Kent	—	3	—	11	14
Surry	—	1	4	1	6
Middlesex	1	6	—	7	14
Westminister	—	17	—	—	17
City	—	5	—	6	11
Total	45	45	17	49	156

* Others mean lands or estates other than manors. These figures, which I owe to the kindness of Dr. Joan Thirsk, are based on the lists contained in her Ph. D. thesis (unpublished).

Thus, it is clear from the facts stated above that the great majority of delinquents consisted of two classes of gentry and nobilities, and that by far the greater part of the lands sequestered and sold by the Parliament was the property of these two classes.

The question now arises as to the purchasers of these sequestered lands. When S.I. Arkhangelsky examined into this problem, he came to the conclusion that the majority of purchasers were "London bourgeoisie" and over seventy per cent of the total purchasers were these bourgeois, army officers and officials of the government of Cromwell. H.E. Chesney came to the same conclution in his study "The Transference of Lands in England, 1640–60". The figures of Arkhangelsky are rather doubtful as they are based on such materials as Composition Papers. As a matter of fact, the task of the sales of the Royalist land was carried out by the Drury House and accordingly, the result of sales is quite different when this sort of records are examined. For example, figures about the purchasers of those estates which lay in the twelve south-eastern counties referred above are, according to the study of Dr. Thirsk, as follows (Table V). In the first place, it must be noticed here that none of those purchasers classified in the table is not so significant in number as Arkhangelsky's conclusion. London merchants and London gentry may be classified as "London Bourgeoisie", but the number of them is 86—only 34 per cent of the total. In the second place, it is important that the Royalists themselves repurchased their sequestered lands through twenty

agents. The number of estates which they repurchased was 41 out of total 142 estates sequestered and sold. It would be possible to prohibit them from repurchasing their lands, but the Parliament was not so eager to do it. We can find in this fact one of the reasons why the English Civil War failed to be a typical bourgeois revolution.

Table V

Purchasers	Number	Percentage
Royalists' agents	20	8
Grantees of land	18	7
London merchants	79	31
London gentry	7	3
Gentry, local & of other districts	79	31
Local yeomen & artisans	41	16
Lawyers	4	1
Unspecified	9	3
Total	257	100

Table VI*

Purchaser	Value of purchased land
Royalists' agents	£23,450
Grantees of land	13,450
London merchants	97,900
London gentry	3,300
Gentry, local & outside	85,900
Local yeomen & artisans	7,800
Lawyers	5,500

* Figures are based on Dr. Thirsk's table, and calculated by price group, taking the median of each group. The purchasers of the lands over £5,000 are assumed, for convenience' sake, to have bought the lands valued just £5,000.

Viewed as a whole, however, most of the purchasers was not the Royalists themselves. The estates formerly owned by 50 Royalists were transferred to the hands of 237 new owners by 1660. Moreover, if we sum up the prices paid by each group for the lands they purchased, it is obvious that London merchants and three groups of gentry bought by far the greatest portion in value (Table VI). Especially, as for the biggest estates over £5,000, five out of ten purchasers who bought lands directly from the Treason Trustees were London merchants, and three out of four purchasers who bought the estates over 5,000 pounds at secound and third hand were also London merchants. Second important group was the gentry—especially 66 local gentry; they bought medium-sized or rather small estates, but the sum of the prices they paid amounted to some £70,400.

III. Land Problems As we have seen above, land transference caused directly by the Civil War was considerably extensive in its scale: Properties once possessed by nobilities or gentry who supported the King were transferred mainly to merchants and local gentry who did not take sides with the Crown. With the lands of Royalists, bishops' lands were also sequestered and sold by the Parliament.

Then, what were the results of such land transference? It seems that one of the most important results was the effect of the Civil War upon manorial right.

(1) Effect of the Civil War on manorial right

In this connection, the most characteristic legislation of the Parliament is perhaps the restoration of court leet and court baron. In 1648, the Parliament decided to reopen the court leet and the court baron of the sequestered manor and thereafter, a "true honest person" appointed by the county committee was to keep the manor court in accordance with the "old custome" of the manor. In other words, Parliamentarians did not aim to abolish the manorial system and to establish modern private landownerships: On the contrary, they wanted to maintain the old manorial custom and to keep the manor court by themselves.

The change brought by this legislation was only that one who kept manor court was no longer the lord or his officials but an official of the Parliament.

The effects of Civil War were, however, far-reaching and penetrated into every manor all over England in such a way as the Parliamentarians had not anticipated. Peasants in every manor accepted the Civil War as the best opportunity to extend their freedom, and

Table VII*

Number of those who	1624	1629	1651	1652	1655
did not appear at manor courts	5	6	35	13	10
offended the Assize of Bread	2	—	11	7	11
offended the Assize of Ale	—	—	15	13	15
violated the custom of commons	1	—	1	7	6
devastated their holdings	—	2	—	-	—
did not repair roads or enclosures	2	—	—	—	6
offended other customs	—	—	1	1	3
Total	10	8	63	41	51

* Figures are based on the records compiled by George Furnham in 'Quarndon Records'.

accordingly, such a remarkable change that the agrarian legislation of the Parliament itself did not aim was brought in every manor: In the first place, number of offenders who violated the custom of the manor increased during and after the Civil War. Table VII shows the number of those who offended the customs of Beaumanor manor (Leicestershire). It will be obvious how frequently the customs were violated during the Civil War. Particularly, it must be noticed in the first place that the number of those who did not make suit at manor courts amounts to 35 persons in 1651, when the sequestration began more enormously. In the second place, it is important two of six offenders who violated the custom of commons in 1655 were peasants who grazed the lord's waste and remaining four were those who erected their cottages in the common ground.

Another example, at Clitheroe in Lancashire, is more interesting. Here, one of the Parliamentary officials was ordered to keep the courts of many manors. But Edward Moseley, the official, was not so eager for his job: In 1651, the county committee wrote to the Committee for Compounding in London as follows....

"You authorized Edward Moseley to keep all courts upon sequestered estates. Some that are beneficial he has appointed his deputies to keep, but all the rest lie unkept and the country cries out for want of them. Let able men be appointed for keeping such courts, and salaries allowed them out of the perquisites of those courts, which otherwise will be lost". The situations were much the same after the Civil War though most of the Clitheroe estates were restored and granted to the Duke of Albemarle. So in 1662, the Duke of Albemarle sent his letter to his steward Charles Molineux, saying "Whereas by the greate negligence of the Steward and Under Steward that have heretofore kept the severall Courts of the Mannors within the Honour of Clitherowe many and greate disorders hathe beene comitted by reason that they seldome or never kept any Courts of Survey but minding their owne profitt more then his majesties advantage they tooke noe care to perfect the Rentalls—in which they were the more incouraged by the late unhappie times wherein his majesties

Government was usurpt and his officers not permitted to excute their offices to put any remedie to those evels which in twenty years have much increased,....". The Duke, at the same time, ordered to keep a court of survey at the next Halmot Court, in order to ascertain how many copyholders did not pay their rents. His steward enquired into this at courts of survey which he kept in several manors, but the results were not so favourable to his lord: For example, in Chatburne manor, there were 50 copyholds of which no rent was paid, but the steward wrote to the Duke that he could not find whom these fifty holdings belonged. Also in other two manors, there were several copyholds of which he could not ascertain the occupiers who had to pay certain amount of rent.

We can find same examples in many other manors. Many lords of manors petitioned to the county committees or the central committee during the Civil War, asking the special order to force their tenants to pay the lawful rent. Manor court was the most important basis of feudal landownership and, since its function became less efficient, it was no longer easy for the lord of the manor to compel their tenants to provide feudal obligation in the same way as before.

(2) Differentiation of the peasantry

As we have seen above, there were some yeomen and husbandmen among the purchasers of sequestered lands. It was rather difficult for them to buy directly from the Treason Trustees as the estates being to be sold were usually too big. Most of the purchasers of peasant class bought at second or third hand, when some of large estates were sold off in smaller parcels.

But this was not so important as to cause the differentiation of the peasantry. More important was the fact that land transference was accelerated as a whole as the result of the Civil War. It was the usual way for Royalists to sell a part of their estates in order to pay the compositions. And also, there were many who sold their lands in the upheaval of the Civil War. Further, it must be remembered that the inefficiency of manor courts caused almost free transference of peasant holdings. Table VIII shows an example at Chatburne manor in Lancashire.

Table VIII*

Peasant holding	1617			1662		
	Number	Area	Rental	Number	Area	Rental
Over 50 acres	—	—	—	1 (1%)	70 (15%)	2/ 8/0 (16%)
25–50 acres	4 (14%)	198 (44%)	5/ 5/4 (33%)	4 (6%)	147 (33%)	4/ 7/10(29%)
10–25 acres	11 (39%)	190 (42%)	7/11/9 (48%)	9 (13%)	138 (30%)	4/ 4/2 (28%)
3–10 acres	8 (28%)	57 (13%)	2/ 7/1 (15%)	13 (19%)	71 (16%)	2/11/9 (18%)
Under 3 acres	5 (19%)	7 (1%)	0/13/0 (4%)	42 (61%)	27 (6%)	1/ 4/10(9%)
Total	28(100%)	452(100%)	15/17/2(100%)	69(100%)	453(100%)	14/16/7(100%)

* Figures are based on the lists contained in 'Court Rolls of the Honor of Clitheroe' (ed. by Wm. Farrer).

It is clearly observed from this table that medium-sized peasant holding from 25 to 50 acres decreased both in number and area after the Civil War, while small holdings under 3 acres increased significantly. On the other side, one of them held 70 acres in 1662. Besides these, three points as follows must be noticed: In the first place, total amount of rent

decreased despite that of area did not changed. This is because the rental of each tenement was not ascertained as the result of disturbance during the Civil War. In the second place, it is observed that the amount of rent paid by the smallest group under 3 acres was comparatively large. In 1662, they held 6 per cent of total area and paid 9 per cent of total rental. Similarly, the group of 3-10 acres held 16 per cent and paid 18 per cent. But two groups over 10 acres held more and paid less. In the third, it is noticed that one of the yeomen in this manor was a draper and held 25 acres of land. It seems that he had some leasehold lands besides these 25 acres. He was a Royalist and paid £50 as the composition for his holdings which was estimated at £30 a year, but his holding in this manor did not decrease even after the Civil War.

Thus, as a whole, it is obvious that differentiation of the peasantry advanced in this manor after the Civil War. There are many similar examples though I cannot show them one by one (More in detail, see my article in the 'Socio-economic History Series', Vol. IV, published by Kobundo).

In conclusion, it will be possible to point out here that the Civil War brought the notable changes in the distribution of landownership as the result of the sale of Royalist lands and that, by far the most important changes, which the agrarian legislation of the Revolution did not include, i.e. the tendency towards the break-up of manor court and the differentiation of the peasantry progressed in most manors.

IV. Agricultural Engineering

Abstracts Nos. 94—100

94. New method for measuring percolation water in paddy fields. Fujio YAMAZAKI, Toshio YAWATA, Noboru NAGATA, Toshio TABUCHI and Shingo IWATA (Journ. Agr. Eng. Soc. Jap. 27, 381-386, 1960)

The authors designed a new method for measuring percolation water in paddy fields. A cylinder which had no bottom, 15 cm in diameter and 10 cm in height was used for the purpose.

After setting the cylinder in the soil, a tip of the tube connected with Mariotte glass tube was inserted into the hole of the cylinder. The water lost by percolation in the cylinder causes a drop of the water level, then, the cylinder is automatically supplemented with water. Diminution of water in the Mariotte tube indicates the quantity of percolation water for that time required.

The merits of this method are;

(1) Percolation can be measured rapidly and correctly. 10 minutes is enough for measuring at one point.

(2) Since the water levels inside and outside of the cylinder are nearly equal while measuring, errors due to the difference in water levels are negligible.

(3) It is possible to measure the percolation water even in paddy fields into which irrigation water flows all the time.

The results which were obtained by this method in newly developed paddy fields on a diluvial plateau showed facts that there was much variety in the amount of percolation at different positions in the field. We assume that this phenomenon may be observed in any paddy fields. In order to improve paddy fields of lower productivity it is important to

evaluate the positional distribution of percolation-rate in paddy fields. For this purpose the above-mentioned method may be very useful.

95. **Percolation of the paddy fields on the upland.** Fujio YAMAZAKI, Toshio YAWATA, and Toshio TABUCHI (Journ. Agr. Eng. Soc. Jap. 28, 137–141, 1960)

On the diluvial plateau often there lie the paddy fields of volcanic ash soil which have low ground water level. Their surface soil and pan layer (which was apparently formed as a result of plowing and other farming operations) have larger volume weight and smaller permeability than the subsoil. In consequence of these physical conditions the irrigation water percolating underground shows negative pressure near the pan layer.

The pan and the subsoil usually contain 15–20% air, so in this case the percolation occurs in unsaturated condition through these layers. The question of whether its air phase is open system or closed system is not clarified yet; it is a subject to be solved in future.

The coefficients of permeability in the pan layer and the subsoil measured in field condition are smaller than those measured in saturated condition. This relation may be explained by the facts that the pan layer and the subsoil contain considerably much air in pores when percolation is taking place and moreover, the volcanic ash soil has much imbibition water which probably can not be moved by percolation.

The pan layer and the subsoil show oxidized condition even in the irrigation season when water always stands on the soil surface.

The field used for this experiment was built four years ago. So this experiment may be considered as a special case because of new field. But we regard the characteristics of percolation described above will hold true even in case of old field. Therefore we consider that the characteristics are not peculiar to new field, but they are the special character of of the paddy fields of volcanic ash soil with low ground water level.

96. **An electrical analogue method for representing a seepage surface of ground water into ditch.** Hiroyuki OGATA (Journ. Agr. Eng. Soc. Jap. 27, 397–402, 1960)

In order to represent the boundary condition of the seepage surface in the electrical analogue method, the author divided the seepage surface into 20 small pieces, and gave each of them proper electric potential by a potential divider. The arrangement of experiment becomes quite easy by this method.

The flow and the distribution of potential will change with the following value;

$$e_n=(v_n/V-h_n/D)$$

where V=electric potential difference between the bottom of ditch and ground surface.
v_n=potential of the n-th small piece from the bottom of ditch.
D=depth of ditch.
h_n=height of the center of the n-th small piece.

To every small piece, such v_n is given that every e_n of the piece may be equal. In case of equidistant ditches which reach the impervious substratum, the relation between flow and e and number of small pieces is given. When the number of small pieces is few, $e=0$ is not always appropriate. The most appropriate value of e may be decided for a certain number of small pieces. The inversion between potential and stream function may easily be made, too. According to such an example of experiment, the experimental value coincided with the theoretical value very well.

97. **Intake test for special apparatus.** Shozo NOGUCHI (Study on the Water Temp. 3, 188–202, 1960)

The experimental apparatus is invented by Mr. K. ITO as shown already in previous number.

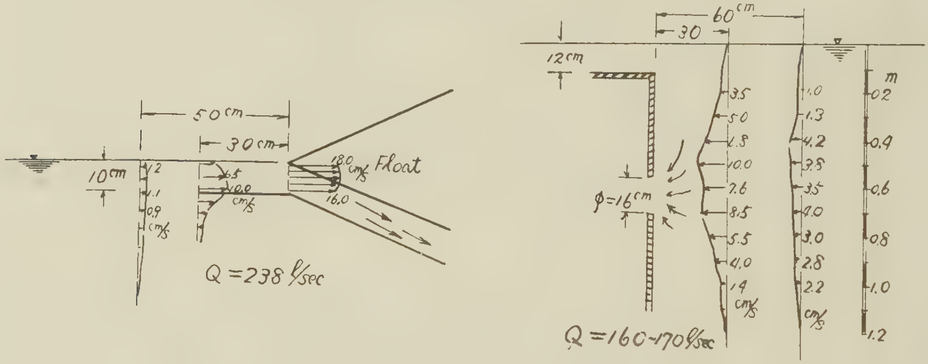
To obtain basic data for the flow of water in the apparatus, experiments were practised in small reservoir having about 50,000 m^3 in volume, 2–6 m in depth.

The obtained results are as follows.

1. Working this apparatus, max. intake water volume is about 240 l/sec, and surface water of reservoir (from water surface till about 15 cm depth) is taken out very smoothly.

2. From the point of view of intake, this apparatus is much better than old type intake apparatus.

3. For example, velocity distribution of intake water is shown as Fig.



(A) ITO's apparatus (B) Orifice type

Fig. Comparison of velocity distribution for intake

4. By this apparatus, velocity of inflow near the float is about 20 cm/sec. But the critical velocity is variable adjusting to the temperature distribution of the reservoir water, or discharge of intake.

98. Study on a new type threshing cylinder. Fusanobu SHOJI (Journ. Agr. Machin. Soc. Jap. 21, 23–26, 1959)

The faults of the present threshing cylinder in Japan are to make so many pieces of ears and straw, and to require more holding force of the bundle in operation. The author has investigated the cause of above faults and devised a new type threshing cylinder, which is made of the cylinder with several collars and many tines, instead of the inverse V-shape tines of the ordinary threshing cylinder.

According to the result of experiment by this new cylinder, it was observed that pieces of ears and straw of rice or wheat in threshing decreased suddenly in comparing the action of ordinary cylinder, but it was also found out, that effectively putting the bundle between collars gets into a slight difficulty and further improvement of cylinder is necessary.

99. Studies on the mole-drain. Part 3. On the durability of mole-drain hole in paddy field. Fusanobu SHOJI and others (Journ. Agr. Eng. Soc. Jap. 27, 119–125, 1959)

According to the result of survey in field, the durability of mole-drains under the paddy field in Japan is diminished by the following main causes: (1) in operating, muddy water flows freely into the mole-drain hole from the cutting slit of edge plate, or flows backward from a tributaty drainage canal; (2) in or after operating, the hole bottom alignment of

mole-drain becomes uneven; (3) livestocks or heavy farm machineries trample down the mole-drain hole from surface of field; (4) Crop roots infiltrate into the mole-drain hole; (5) crawfish or other small animals barrow their way into the mole-drain hole.

Analytically speaking authors obtained the following results from experiments in field: (1) directly after operation, the ordinary cross-section of mole-drain hole becomes a vertically long ellipse and its cross-sectional areas are small in the cases of deep drains, small consistency index of subsoil and small soil hardness; (2) several years after operation, silt accumulates at the hole bottom and the cross section of mole-drain hole becomes a laterally long ellipse or a crescent. Soil property of silt at hole bottom is slightly less in percentage of particle-size from 0.12 to 0.01 mm as compared with subsoil.

When the subsoil is composed of an equal ratio of clay, silt and sand, the accumulation of silt is comparatively small. This results is quite different from the theory of Prof. NICHOLSON in which he says the more clay content, the longer becomes the durability of mole-drain in upland field. It is thought that this perhaps depends upon high water content of subsoil owing to irrigation for paddy field.

100. **Transistorized tele-torquemeter.** (2). Yoshichiro YASUDA and others (Ann. Rep. Eng. Res. Inst. Fac. Eng. Univ. Tokyo, 18, 1-3, 1959)

Three channels of tele-torquemeter were constructed with some partial improvements on the previous barrack set and field experiment was also carried out. Namely, previous slope detector was changed with Foster-Seeley detector for better linearity and all of variable resistor in the mobile unit were replaced by fixed resistors and rotary-switches from the view-points of mechanical vibrations caused by moving tractor and engine. Cross talk was considered to be 40 db or more and disturbances due to mechanical vibrations and ignition noise from the engine were observed to be negligible. Sufficient linearity of overall characteristics was attained and threshold of measurable torque was found to be Kg-m. The whole system of tele-torquemeter is expected to be accomplished as soon as possible.

V. Fisheries

Abstracts Nos. 101—122

101. **An idea on the maximum permissible concentration of radioactive materials in sea-water.** Yoshio HIYAMA (Rec. Oceanogr. Works in Japan, 5-2, 98-104, 1960)

Knowing the total body burden or amount in a certain organ of human body about various kinds of elements, and knowing the concentration of these in normal sea-water, the author tried to calculate the maximum permissible concentrations of several radionuclides in seawater by using the idea of balance between the two in the state of equilibrium.

102. **Sr^{90} in marine organisms in Japan.** Yoshio HIYAMA, Makoto SHIMIZU, Junko MATSUBARA, Tamiya ASARI and Ryushi ICHIKAWA. (Radioactive Contamination of Marine Products in Japan. Document for the Scientific Committee on the Effect of the Atomic Radiation of the United Nations, 9-12, 1960)

The tendency of increase of Sr^{90} level in clam shell and bone of varions fishes obtained by the continuous survey since 1954 to 1959 was reported.

103. Predation of rain-bow trout fry traced by radio-isotope. Yoshio HIYAMA, Yukio NOSE, Makoto SHIMIZU, Junko MATSUBARA, Koshi ONODERA, Yasuo KODAMA and Norio SUZUKI (Bull. Jap. Soc. Sci. Fish. 26, 863–867, 1960)

ONODERA and others have investigated the reduction of number of trout fry after liberation since 1957, but the causes of the reduction were not known yet. In 1959's investigation, HIYAMA and others joined to assure the role of predation by using radio-isotope. Among 2034 individuals of rain-bow trout fry liberated (small size group 1.2 gr., large size group 2.7 gr. in average), just a half of them was injected at back muscle by $^{65}ZnCl_2$ solution (1 μc/0.03 cc per individual). No mortality ratio, nor growth was affected by treatment during 2–3 months, while radio-activity remains distinctly above background, by external survey by G-M-survey meter. Water, soil, plants and animals in the vicinity were surveyed frequently after the liberation, but no radio-activity was found except adult trout survived since last year and the excrete of a bird (*Cinclus pallasii*).

Feeding adult trout by radio-active trout fry powder in aquarium, ratio of up-take ($f_1=0.24$) was measured. Getting Zn^{65} amount in adult trout body caught in the stream (x cpm) and that in fry at that time (a_i), the number of trout fry taken by an adult trout (y) can be obtained by the formula ;

$$y=\frac{x}{f_1}\times\frac{2}{a_i}$$

Substituting pigeon for *Cinclus pallasii*, the excretion ratio (amount of Zn^{65} in excrete in a day/amount of Zn^{65} in trout fry fed in a day), $f_2=0.84$, was obtained. By getting the average amount of Zn^{65} in an excrete of a bird ($\bar{x}=100$ cpm), and getting information on frequency of excrete (N) (126 times a day by a couple of bird) from Mr. Chuichi ISHIHARA, the number of trout fry taken by a couple of bird in a day (y) can be obtained by the formula :

$$y=\frac{N\bar{x}}{f_2}\times\frac{2}{a_i}=20$$

By these informations, 57 days after liberation when the last adult trout was taken (11 ind. altogether), it was enumerated that 33.5 individuals of fry were taken by adult trout, and 1140 by a couple of bird nested near the liberation point. On the other hand, ONODERA and others enumerated that 782 ind. of trout fry survived at that day by test fishing. So, the reduction of trout fry since liberation was $2034-782=1252$ individuals. Among them 94% was caused by predation by eleven adult trouts and a couple of bird, and 96% of predation was by the latter.

104. Stopping effects of electric fish screen. Takaya KUSAKA (Bull. Jap. Soc. Sci. Fish. 25, 269–274, 1959)

The author has done to see stopping effects of electric fish screen in place of leading net. Fish materials used were 10 *Carassius auratus* Goldfish of total length 5.0 cm., and 10 *Oryzias latipes* Japanese Killifish of T. L. 2.5 cm. as both live in fresh water, and 12 *Sphaeroides niphobles* Japanese common puffer T. L. 5.0–7.0 cm. in sea-water.

Two electrodes are set in the upper and lower sides at the center of tank, and were closed alternate electric current of sine wave of 50 cycles regulating its voltage and amperage by transformers (Fig. 1).

Frequencies of fish individuals passing through the center of tank were compared on cases closed and off the electric current.

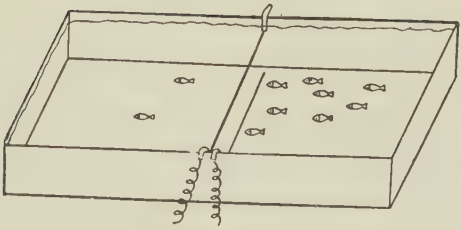


Fig. 1 The set for electric fish screen

The results of each fish materials are taken efficiencies of passage and those significancy of stopping effects were judged (Fig. 2)

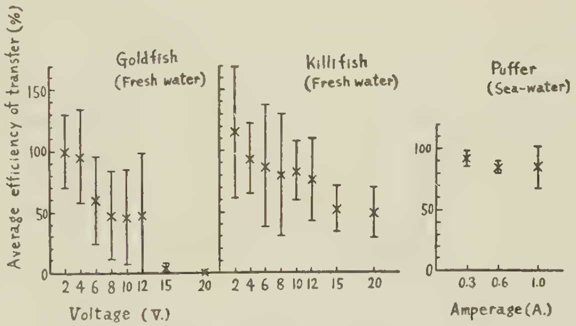


Fig. 2 Stopping effects of the electric current in fresh water and sea-water.

In general, stopping effects of electric current in fresh water is about 50% on better condition, as if sufficiently available. On the other hand, the case of sea-water is about 20% and also, less availability, because it is required several hundreds times of amperage than the case of fresh water.

105. An experiment on the fin-clipping method for estimating the population size of eel in a culture pond. Yukio NOSE (Bull. Jap. Soc. Sci. Fish. 25, 275–281, 1959)

The number of eel, *Anguilla japonica* in a culture pond (about 1 acre) was estimated by PETERSEN mark-and-recapture method, and the estimates which were obtained from the first set of data and that of the first and second sets of data, treated as a whole, were compared with the actual number of eel captured after the draining of the pond.

The results indicated that the estimates were fairly greater than the actual number of fish (Table) and the source of error was attributable to misjudging some of the marked fish for those that were unmarked, which is caused by the rapid regeneration of the clipped fin (right pectoral fin) in some of the marked fish.

The correction equation for p, marked ratio in samples, was derived as follows,

$$p=\frac{p'-\varepsilon_2}{1-\varepsilon_1-\varepsilon_2} \tag{1}$$

and $V(p)$, the variance of p was as follows,

$$V(\hat{p})=\frac{(p'-\varepsilon_2)(1-p'-\varepsilon_1)}{n(1-\varepsilon_1-\varepsilon_2)^2} \quad (2)$$

where p' is the apparent marked ratio in samples, p is the marked ratio in samples when there is no misjudgement, ε_1 is the rate of misjudging the marked as the unmarked, ε_2 is the rate of misjudging the unmarked as the marked, which is caused by deformity of the fin, and n is the size of samples.

In the present experiment, ε_2 was neglected and ε_1 was about 0.2. The recalculation, using the above equations, showed the new estimates were both in conformity to the actual number of fish (Table).

Table. Comparison of the estimates with the actual number of fish in the pond. $\hat{N}$, the estimate of population size, $\hat{N}_L$ and $\hat{N}_U$, the lower and upper limit of 95% confidence interval.

	No.	$\hat{N}$	$\hat{N}_L$	$\hat{N}_U$	Probability distribution used.
PETERSEN's estimate	1	4871	4053	6105	Poisson.
			4075	6055	Binomial.
			4192	5811	Hypergeometric.
	2	4506	3951	5242	Poisson.
			3968	5213	Binomial.
			4181	4866	Hypergeometric.
Actual number of fish			3833–3933		
New estimate	1	3605	3219	4095	Binomial.
	2	3899	3322	4717	

106. The feedidg mechanism in some young fishes. Fujio YASUDA (Rec. Oceanogr. Works in Japan, 5-2, 132–138, 1960)

In this work, the author made a study of the feeding mechanism that takes place before the filtering of food by gills in plankton-feeders and before the trapping of food by the mouth cleft in carnivorous fishes.

In another report, the author said that the cleft does the function of trapping and the mouth breadth determines the size of food. Taking this to be true a comparative study on the development of the cleft and the mouth breadth was made.

As the materials, four marine fishes, red barracuda, mackerel, horse mackerel and anchovy and three tropical fishes, ticto, sumatra and blue gourami were studied. The results obtained are reported here as follows:

1. In the feedidg mechanism of fish in their young stages, the mouth structure, especially the cleft and the breadth are very important factors.

2. In the development of the cleft and the breadth there can be observed two types. One, where the cleft length and the breadth cross at some stage after hatching. The other, where the crossing does not occur or is not clearly visible.

3. In the first type, during the stage before the crossing has occurred, the fish is not active in its feeding habits and only feeds on inactive micro food particles. However, the other type begins feeding actively immediately after hatching.

107. **The feeding mechanism in some carnivorous fishes.** Fujio YASUDA (Rec. Oceanogr. Works in Japan. 5-2, 153-160, 1960)

The feeding habits of carnivorous fish (especially the trapping of prey) is mostly controlled by the structure of the mouth, which thereby limits the size of the prey.

The feeding habits and relationship of prey size and mouth structure of four important carnivorous fishes, mackerel, big-eye, yellowtail or amberjack and red barracuda and for comparative study the anchovy, a true example of a plankton-feeder found in the coastal waters of Japan were studied by the author and the following results obtained are reported here;

1. In the feeding mechanism, namely the trapping of prey, the cleft of the predator serves the most important function, and the mouth breadth limits the size of the prey.

2. As regards the size of the prey, the body depth or body width is more important than the total length or body weight of the prey to the predator in case of their trapping.

3. The "proper size" of the prey (body depth or body width) is that size which is almost equal to the size of mouth breadth of the predator.

108. **The types of food habits of fishes assured by stomach contents examination.** Fujio YASUDA. (Bull. Jap. Soc. Sci. Fish. 26, 653-662, 1960)

The stomach contents survey of the fishes is an indispensable research method to investigate the feeding habits, the living area of fishes and also the ecological and physiological relation between each species.

The research method must differ according to its purpose, but generally the survey begins from the classification of the food organisms in the stomach contents of fishes, and then extends to the quantitative analysis such as the weight of food, the volume of food and the digestive degree of food.

Here, the author is going to report the results of examination of stomach contents of 73 species of fishes which were caught mainly at the coastal area of Kanagawa Prefecture of Japan by set net, boat seine and "shirasu biki" (one of the boat seines which catch the larval fish, particularly sardine species) during 1957 to 1958.

The stomach contents of almost every individual fish of all the species obtained were examined qualitatively. Among them of the species the number of individuals was so sufficient that, the author examined quantitatively to see the individual variation.

From these results, the author could know some remarkable tendency of the variation of the stomach contents of these fishes.

The results obtained are summarised, as follows:

1. The surveyed fishes were classified into four groups, and the piscivorous group was also divided into the particular feeding group.

2. The amount of food eaten by these fishes were classified into three types, such as plankton-feeders, omnivorous and some carnivorous fishes and carnivorous fishes especially piscivorous fishes, and each feeding group belongs to different types.

3. The rate of occurrence of empty stomach is high in the adult fishes and also high in the high food order fishes.

109. **The relationship of the gill structure and food habits of some coastal fishes in Japan.** Fujio YASUDA (Rec. Oceanogr. Works in Japan, 5-2, 139-152, 1960)

The gill in fishes, in the case of plankton-feeders are important organs for filtering food.

It has been said that the structure and number of gill rakers differ with different fishes, and the more active the feeding habits of the fishes are, the less developed the gill rakers are. There have also been cases where the gill rakers are absent and in all such cases the

fish have been found to be very active feeders, feeding on anything.

The author in this study examined the developmental changes of the gills and its relationship to the food habits of the fish in 21 species of coastal fishes of Japan.

110. A study on the growth and rearing methods of the fry of black porgy, *Mylio macrocephalus* (Basilewsky). Shogoro Kasahara, Reijiro Hirano and Yasuo Ohshima (Bull. Jap. Soc. Sci. Fish. 26, 239-244, 1960)

Hitherto, many attempts have been made to rear the larvae hatched from the pelagic eggs of various kinds of marine fishes. However, notwithstanding the efforts made by many biologists and fish-culturists, no satisfactory method to raise the fry and to maintain their survival at a high percentage has yet been achieved.

The authors made three trials in the artificial fertilization of the black porgy, *Mylio macrocephalus*. These three trials were carried out at the Iride fish market, situated on the western coast of Hamana Bay, on the 30th of April, the 7th of May and the 23rd of May, 1959.

Rearing experiments were carried out at the Ikawazu Fisheries Laboratory, Aichi Pref., to which the fertilized eggs were transported from there.

The eggs hatched out in 40-50 hours and 60-70 hours at water temperatures of 19°C and 17°C respectively. As food for the hatched out larvae, substantial amounts of several kinds of protozoa were previously cultured and given to them from a few days after hatching. It was observed that the fry, shortly before the yolk sac was entirely absorbed, namely at the age of two to three days after hatching, began to feed on fine Dinoflagellata, *Oxyrrhis* sp. ($10\text{-}30\mu \times 8\text{-}15\mu$). Subsequently, at fry measurements of 3-3.5mm. in total length, a species of Spirotricha, *Stylonchia* sp. ($50\text{-}100\mu \times 30\text{-}40\mu$) were largely fed on by the fry. Later, as the fry grew, they were observed to take in various living food organisms of gradually larger size in direct relation to their growth. For instance, in our experiments it was observed that the fry took the nauplius of copepoda or barnacle at 4-4.5mm. in total length, i. e. 9-12 days after hatching; the nauplius of brine shrimp at about 5mm. in total length, i. e. 15 days after hatching; young mysid shrimp at about 7mm. in total length, i. e. 3-4 weeks after hatching and finally the fry was able to feed on chopped fish when they measured about 15mm. in total length, i. e. 6-7 weeks after hatching.

As to the number of fry that survived, we obtained 638 young porgies (13% to the number of the hatched larvae) after 52 days in the first round of rearing, 614 fry (17.5%) after 45 days in the second trial and 102 fry after 60 days in the last trial. In each trial to raise the larvae, a considerablly high mortality occurred at the age of 6-8 days after hatching. It is observed that these days are the period when the fry change their diet from protozoa to larger food organisms.

Further study is necessary so as to make it possible to improve the survival rate of the fry especially during the period above-mentioned. Once this critical period was crossed it was observed that the fry grew rapidly, reaching about 4.5mm. after two weeks, 10.1mm. after a month and 32.0mm. (1.03gr. in body weight) at the age of two months.

111. On the spawning of the fish "Ayu", *Plecoglossus altivelis* T. et S. I. The structure of spawning population and the spawning behaviour. Rikizo Ishida (Bull. Jap. Soc. Sci. Fish. 25, 259-268, 1959)

As to the sex ratio of the population of the fish, "Ayu", crowding at a spawning bed, it has been known that in the early period of the spawning season (from September to November) males predominate, while, towards the end of the season females predominate.

But no attempt to solve this phenomenon has been made yet. In this study investigations were made about the change of the sex-ratio during the spawning season in connection with the maturity, size of population and numbers of eggs laid on the spawning bed in Sagami River, Kanagawa Prefecture, and on the other hand, the spawning behaviour of "Ayu" was observed at the artificial bed prepared in experimental ponds.

The results obtained are summarized as follows:

1) The rate of males in a population increases gradually from the early period to the middle of the spawning season, where reaching the climax of spawning, and thenceforth it gradually decreases.

2) There is found a direct proportional relation between the rate of males and the size or the maturity of population. Furthermore the number of eggs laid on the spawning bed has a tendency to be the largest when the rate of males reached the maximum.

3) The matured males crowd at the spawning bed. As a result the silt which cover the gravels on the bed are cleared away. Then, a few attendant females join to the male group and the spawning is carried out. Thus, it may be said that this males' crowding is, so to speak, a prespawning behaviour and the occurrence of high rate of males in the climax of the spawning season is closely related with such a spawning behaviour.

4) During the spawning process gravels on the bed are sifted by the fish, thus a circular hollow being formed, and there is also a high correlation between the amount of gravels sifted, which indicates the intensity of spawning activity of fish, and the number of eggs found on the bed.

112. Notes on the sampling gears and the animals collected on the second cruise of the Japanese expedition of deep seas (JEDS-2). Yasuo SUYEHIRO and others (Oceanogr. Mag. 11, 187–200, 1960).

During the period from the 5th. to the 30th. September in 1959, the second cruise of the Japanese expedition of the deep seas was carried out at the north region of Japan Trench. The benthic and mid-water animals were collected with several kinds of gears, a beam trawl, two sorts of dredges and two sorts of plankton nets. In this report, we gave a detailed explanation of the sampling gears and a provisional statement of the deep sea animals collected. The collecting stations were as follows:

St. S : Off Onagawa near Sendai City, 37°55′N, 143°25′E, 2,350–2,230m, rectangular dredge and beam trawl.

St. A_2 : 40°25′N, 144°36′E, 6,790m, triangular mid-water plankton net.

St. A_3 : 40°59′N, 147°26′E, 5,290m, triangular mid-water plankton net and 130cm conical net.

St. B : Off Kushiro City, 42°40′N, 144°30′E, 490m, Niino's dredge.

St. C_2 : 42°46′N, 148°10′E, 8,677m, triangular mid-water plankton net.

The benthic metazoan animals obtained are classified provisionally into 119 forms, and they amount 770 in total number. The details of systematical grouping and number of species are shown in two tables.

An outline of quantitative data on deep water planktons collected at each haul is also given in another table. Considering the fact that the sampling gears were used for the first time in deep sea collections, we can say that the results were all very satisfactory. However, several points for improving the nets were found.

113. A chytridean parasite on the *Porphyra*. Seibin ARASAKI (Bull. Jap. Soc. Sci. Fish. 26, 543–548, 1960)

A chytridean parasite, presumably a new species belonging to *Olpidiopsis*, was found on the frond of cultured *Porphyra* (Asakusa-Nori) at several culture fields especially in the north-eastern region of Japan. The name of "the cytrid disease of *Porphyra*" was given to the disease caused from the present fungus. The fungus attacks not only the mature frond but also the young, germling and free spore of the *Porphyra*. Although the life-span of the individual fungus is rather short, no more than a few days, yet it appears one after another generation for almost all harvest season covering from November till the end of March. The extent of the damages of the disease has not been distinctly known, but it seems not to be so great for the mature frond, but to be severe and fatal for the young *Porphyra*.

The fungus grows intracellularly in the living cell of the host. It is colorless, globular or ovoid in shape, and 7.5–13μ (mostly 9.5–10μ) in size, and contains a few minute oily granules. In maturity it produces 4–6 planospores, and protrudes out a short exit tube through which the spores are discharged one by one. The spore is colorless, reniformal or irregular form, and has two similar flagella on the ventral side. It swims about without showing any phototactic activity for a certain minutes, then loses the flagella, and becomes rest. The resting spore is spheric, 3–5μ (mostly 4.5μ) in its diametre, and attacks directly the living cell of *Porphyra*.

On the other hand, the late Dr. YENDO (1919) reported the male and female planogametes produced on the filamentous germlings of the carpospores of *Porphyra*. It seems to be most probable that he had took the present chytrid and its spores for the planogametes of *Porphyra*.

114. An ecological study of *Undaria pinnatifida* SUR. IV. On the influence of desicccation upon the resting zoospores, the gametophytes and the young sporophytes. Yunosuke SAITO (Bull. Jap. Soc. Sci. Fish. 26, 13–20, 1959)

For the purpose to test its ability to stand to desiccation, the resting zoospores, the gametophytes of various growing stages, and the young sporophytes of *Undaria pinnatifida* were respectively exposed to the air of various humidity (45, 60.7, 70.4, 84 and 100%) for various duration (15, 30, 60, 150, and 180 min.) at different air temperature.

After the necessary treatment they were immersed again into the normal sea water and then cultured for two or four days when their vitality or survival rate, growth rate and condition of health were examined and estimated under microscopical observations.

The results obtained were summarized as follows:

(1) When they were well dried in the air the resting zoospores, all cells of the gametophytes and the young sporophytes were shrivelled and died with the appearance like 'plasmolysis' in 5–6 minutes without regard to temperature and humidity of atomosphere.

(2) The resting zoospores were more sensitive to desiccation than gametophytes and the young sporophytes. In the case of gametophytes, those which were growing actively were more sensitive than those in resting stage.

(3) The resting zoospores, the gametophytes and the sporophytes could alive in the air of 100% humidity for long time, but in the air of lower humidity than 70.4% the resting zoospores were fatally affected in about 30 minutes and the gametophytes and the young sporophytes were affected in respectively 1 and 3 hours.

However the time which needs for desiccation of the materials varied with the temperature, that is to say, when the temperature is higher, the time becomes shorter. Besides, the time varied whether they were cultured purely or not, i.e. it took much time when the glass slides were contaminated with other organisms such as diatoms etc.

V. **On the shape of cultured fronds.** 1. Yunosuke SAITO (Bull. Jap. Soc. Sci. Fish. 26, 250–258, 1960)

It has been widely accepted that the species *Undaria pinnatifida* SUR. has included two different forms, f. *typica* and f. *distans*. However, the necessary characteristics to identify these two forms have not adequately been clarified.

This study was carried out for the purpose of finding out the fittest characteristics.

The materials and the method of investigation are as follows:

(1) The materials were collected from the fronds which were introduced from various original habitats and cultured by hanging-method in Toyohama Harbour of Chita District in Aichi Pref., and also from the fronds in natural habitat at Toyohama and Ōno, in the same district.

(2) On these materials, biometrical investigations were made on several portions of the same frond, such as the length of frond (L_1), the length of stipe (L_2), the distance from the lowest margin of blade to the upper end of sporophyll-part (L_3), the width of frond (W: the broadest portion in young fronds; W_1: the broadest portion of pinnate blade in adult fronds; W_2: the same of basal part of blade), the number of pinnate blade (N_1), the number of wrinkle of sporophyll (N_2), and the shape of sporophyll etc.

Then, these numerical values and interrelations between some of them were compared among the cultured fronds introduced from three original habitats (Toyohama, Ōno and Oginohama, Miyagi Pref.), those between the wild and cultured fronds and also those between the fronds of the first and second generations.

The results obtained may be summarized as follows:

(1) Obvious differences were recognized among the cultured fronds of above-mentioned three habitats on the following items: the ratio of L_2/L_1, the relationship between N_1 and L_1, the number of wrinkle of sporophyll and the growth rate of frond.

(2) As to the comparison of the wild and cultured fronds at Toyohama and Ōno, only a few differences were recognized. They are namely, the ratio of W/L_1 and the length of frond at the time when the first wrinkle of sporophyll was formed, in the Toyohama-specimens, and the ratio of L_2/L_1 in the Ōno-specimens.

As to the difference between the frond of the first and second generation, it was not recognized in the fronds of Toyohama, but was imperceptively seen on the ratio of L_2/L_1 and the same of W_2/W_1 in those from Oginohama.

(3) Consequently, it was suggested that the shape of fronds at various habitats were distinguishable especially in such characters as follows: (a) the ratio L_2/L_1, (b) the relationship between N_1 and L_1, (c) the length of frond at the time when the wrinkle of sporophyll was formed, (d) the relationship between N_2 and L_1, (e) the number of wrinkle of sporophyll, (f) the growth rate of frond. Besides these marked characteristics were scarcely lost by the culture and also were reiterated for some, at least two, generations.

115. **Change of free amino acids during the manufacturing process of "Katsuwobushi" (dried bonito).** Shōji KŌNOSU and Yoshiro HASHIMOTO (Bull. Jap. Soc. Sci. Fish. 25, 307–311, 1959)

Katsuwobushi is a dried product made from bonito meat by boiling, sun-drying and moulding processes and used for stock. From the view point of its taste, the effect of these processes on free amino acids was investigated by analyzing the extractives of each step product.

The raw material revealed a very high value of histidine (5018 mg% on dry basis), which

occupied about 80% of total free amino acids. The content of taurine (564 mg%), lysine (261 mg%) and alanine (144 mg%) was also found to be comparatively high, whereas aspartic acid, cystine, tryptophan and arginine were not detected. A portion of each amino acid was lost in the boiling process. During the smoking process, some amino acids increased slightly, whereas others remained almost unchanged, and most of the amino acids decreased again in the course of moulding, perhaps in its earlier stage, judging from the changes of amino-N.

There have been some discrepancies among the reports on the change of free amino acids in the moulding process, but the present results do not support the opinion that the flavor of Katsuwobushi is improved by the increase of some amino acids in the moulding.

116. **Inosinic acid contents of foodstuffs. Part 2. Katsuwobushi (dried bonito).** Takao FUJITA and Yoshiro HASHIMOTO (Bull. Jap. Soc. Sci. Fish. 25, 312–315, 1959)

The change of nucleotides during the manufacturing process of Katsuwobushi was examined on the same specimens described in the previous paper (Bull. Jap. Soc. Sci. Fish., 25, 307–311, 1959)

Inosinic acid in the raw material amounts to 822 mg%, the value corresponding to about 90% of the total acid soluble nucleotides, which contain only small amounts of adenosine mono-, di-, and triphosphates. Inosinic acid is lost to some extents during the manufacturing process, mainly in boiling and smoking process, but it is fairly well retained in the final products. Adenosine triphosphate, on the contrary, disappears in the process.

The results of determination on various kinds of commercial product show that the inosinic acid content varies from 279 to 915 mg% with samples and has no definite relation with the place of production or the grade of quality. These value are much higher than those reported thus far. The histidine content estimated on six specimens ranges from 2.8 to 3.9% on dry basis and the molar ratio of histidine to inosinic acid from 9 to 25.

117. **Evaluation of inosinic acid and free amino acids as tasting substance in the Katsuwobushi stock.** Shōji KŌNOSU, Yasuhiko MAEDA and Takao FUJITA (Bull. Jap. Soc. Sci. Fish. 26, 45–48, 1960)

The role of inosinic acid and free amino acids in make-up of the taste of Katsuwobushi stock was surveyed by sensory test. Several kinds of test solution were prepared on the basis of the analytical values of these ingredients reported in the previous papers and tested by a panel of about twenty judges in paired comparisons.

The combination of inosinic acid and amino acids was judged to be more delicious than inosinic acid alone and to have a distinct meaty flavor fairly similar to that of the Katsuwobushi stock. The amino acids, on the other hand, were almost tasteless in the concentration tested, but they clearly exerted an enhancing effect on the taste of inosinic acid. The most abundant amino acid, histidine, revealed no such an effect, and the taste became considerably weak, if the other amino acids were omitted, though they were rather scanty in quantity. It seems probable from these results that the greater part of delicious and meaty flavor of the stock is attributed to inosinic acid, enhanced by the amino acids other than histidine.

No conclusive judgement was obtained in the comparison between histidine inosinate and sodium inosinate, and this disagrees with the KODAMA's view that the organic salt is more delicious than the inorganic one.

118. **Two hemoglobins in chum salmon.** Kanehisa HASHIMOTO and Fumio MATSUURA (Nature, 184, 1418, 1959)

Components F and S of chum salmon hemoglobin (Hb) were crystallized by a fractional

crystallization method using ammonium sulfate. Both preparations, after recrystallizing twice, were found to be practically homogeneous electrophoretically. They were then submitted to measurements of sedimentation and diffusion constants in a phosphate buffer of 0.1 M, pH 7.0, and frictional and axial ratios and molecular weight were calculated using these constants. According to the results, the constants and ratios of component F are in a good agreement with those for mammalian Hb's, whereas of component S not. For example, the axial ratio of component S is more than double that of component F, namely, component S 10.2: component F 4.6 as prolate or S 12.8: F 4.8 as oblate. However, the molecular weights of both components seem not to be different essentially from each other.

Another difference was observed also in the functional property between them. Oxygen dissociation curve was measured in phosphate buffers between pH 7.75 and 6.6 for both components separated from hemolyzate by starch block zone-elcetrophoresis. The effect of pH on the oxygen equilibrium was observed clearly for component F, whereas scarcely for another component. The oxygen affinity of component F was much lower than that of component S.

119. Studies on the discoloration of fish meat during the freezing storage. Part 2. A spectrophotometric method for the determination of ferrous and ferric forms of myoglobin in tuna meat. Yoshihiko SANO, Kanehisa HASHIMOTO and Fumio MATSUURA (Bull. Jap. Soc. Sci. Fish. 25, 285–289, 1959)

The method reported previously on pure myoglobin solution (SANO et al., this Bull. 24, 519–523, 1958) was found to be also applicable to extracts from tuna meat. The procedure is as follows: 10 g of the finely ground meat are weighed into a glass-stoppered centrifugal tube and extracted for about 2 hours with 20–25 ml of distilled water with occasional shakig in atomosphere of carbon monoxide. After centrifugation, 15 ml of the supernatant are taken into another glass-stoppered centrifugal tube, to which an adequate amount of 1 N NaOH solution is added for removing cloudiness, and the solution is left standing at least for one hour in a refrigerator. After centrifugation, 5 to 10 ml of the supernatant are diluted with 10 ml of the phosphate buffer of 0.2 M and pH 7.0 and divided into three portions. These portions are subsequently treated as described previously (*loc. cit.*) and the percentage proportion of metmyoglobin to the total myoglobin in the extract is calculated. The reliability of this mehod was sufficiently high as checked by the recovery tests.

120. Comparative studies on two hemoglobins of salmon. Part 2. Crystallization and some physical properties. Kanehisa HASHIMOTO and Fumio MATSUURA (Bull. Jap. Soc. Sci. Fish. 25, 465–469, 1959)

The two hemoglobin components of chum salmon were successfully crystallized by the following method: the hemolyzate was saturated with carbon manoxide, fractionated with ammonium sulfate, and the fraction between 35 and 60% saturation at neutral pH was taken out and dissolved in distilled water. The solution was then made turbid slightly with the same salt and left standing overnight at a cold place, until trapezoid crystals of component F appeared. The solution from which the crystals of component F were filtered off, was dialyzed against tap water to denature remaining component F. After centrifugation, the supernatant was reduced with sodium dithionite, saturated with carbon monoxide, and rendered slightly turbid with the ammonium salt at about pH 6.3. After standing for two to three days at a cold place, clusters of needle-shaped crystals of component S were observed in the solution. The preparations of both components obtained thus were proved, after

twice-recrystallization, to be practically homogeneous by moving boundary electrophoresis and other methods.

The crystalline specimens were then analyzed for some physical properties such as sedimentation and diffusion constants and both components were found to be different appreciably from each other in these constants and the frictional and axial ratios calculated using these constants, although they seemed not different in the molecular weight.

121. **Multiple hemoglobins in fish. Part 2.** Kanehisa HASHIMOTO and Fumio MATSUURA (Bull. Jap. Soc. Sci. Fish. 26, 354–360)

It has been shown previously (HASHIMOTO et al., this Bull. 24, 719–723, 1959) that some species of fish possess multiple hemoglobins (Hb's). In order to clear up the significances of this phenomenon, further experiments were performed for several species of fishes from sea-, fresh-, and brackish-water. According to the results, the fishes examined were found to have one (goby, mullet, and carp), two (black bream, *Inimicus* sp., loach, chum, red salmon, and Hime-masu), three (dolphin fish, rainbow and brook trouts) or four Hb components (pink and king salmons) in electrophoresis. In the heat coagulation test, all the Salmonidae fishes showed the presence of two components of Hb, whereas the other species apparently only a single component.

Both electrophoretic property (the number of components, and their mobilities) and the property relating to heat coagulation (the position and shape of the coagulation curve, and the number of components) are more or less species-specific, though some common features may be pointed out fairly strongly among closely related species such as the Salmonidae fishes examined here. These properties may be, therefore, used for classifying these fishes or other fishes.

122. **Some properties of cytochrome c from *Porphyra tenera*.** Kanehisa HASHIMOTO and Ikuko MASUHIRO (Bull. Jap. Soc. Sci. Fish. 25, 561–564, 1959)

Cytochrome c (Cyt) was purified from a red alga, *Porphyra tenera*, by the YAKUSHIJI's method with some additional modifications such as the application of starch block electrophoresis and the treatment with lead acetate. This preparation was fonnd to contain 0.47% iron and to be homogeneous electrophoretically at various pH values examined. The visible absorption spectra of this Cyt showed some characteristics, especially in reduced form; in 0.1 M acetate buffer of pH 6.0, the extinction coefficient of the α-maximum (at 553 mμ) was only three fourths of that of vertebrate and as indicated already, the spectrum shifted towards longer wave lengths than that of vertebrate; in 0.2 N NaOH the whole spectrum became very similar to that of vertebrate, and the extinction coefficient of the α-maximum notably increased. Such a large difference of spectrum as caused by the pH change has not been reported for this pigment from any sources. The pH *vs.* mobility curve of this algal Cyt differed also remarkably from that of vertebrate. Its isoelectric point was found to be around pH 4, which seems very specific considering that those for vertebrate Cyt's are reported to be above pH 10.

VI. Forestry

Abstracts Nos. 123—138

123. **The *Pteridophyta* in the Izu Peninsula, Central Japan.** Satoru KURATA, Kenzô SATAKE and Susumu MASHIKO (Tokyo Regional Forestry Office, 1–45, 1960)

An enumeration of 241 species and 16 varieties is offered. Among them, 41 species or varieties have their northern-most habitats in this peninsula. Above all, it is very interesting that such subtropical genera as *Angiopteris*, *Antrophyum*, *Colysis*, *Elaphoglossum*, *Histiopteris*, *Lindsaea*, *Nephrolepis* and *Plenasium* have their northern-most habitats there. From this fact, it is conceivable that there is an important demarcation line regarding southern elements between this peninsula and the Bôsô Peninsula, the next northern step on the Pacific side of the Japanese Archipelago. The significance of ferns as the indicator plants is also considered. For example, in the warm temperate part of this peninsula such ferns as the *Polystichum polyblepharum* group, the *Diplazium hachijoense* group and *Athyrium otophorum* are good indicators of the sites suitable for afforestation of *Cryptomeria*, and in the cool temperate part *Polystichum retroso-paleaceum* var. *ovato-paleaceum*, *Diplazium squamigerum* and *Athyrium Vidalii* are such indicators.

124. Notes on Japanese ferns (20). Satoru KURATA (Journ. Geobot. 8, 80–86, 1960)

From the taxonomical and geobotanical view-points, critical notes are written on such ferns as *Athyrium eremicola* (sp. nov.), *Athyrium pseudo-iseanum* (hybr. nov.), *Diplazium fimbriichlamys* (a southern type of *Diplazium japonicum*), *Diplazium Tetsu-Yamanakae* (sp. nov.) and *Diplazium Toriianum* (hybr. nov.) and *Diplazium virescens* var. *okinawaense* (new to Hong Kong).

125. Butt-rot of *Tsuga Sieboldii* and *Tsuga diversifolia* in the Tokyo University Forest at Chichibu. Shun-ichi YOKOTA (Misc. Inform. Tokyo Univ. Forests, 13, 12–18, 1960)

The butt-rots of *Tsuga Sieboldii* and *Tsuga diversifolia* in the Tokyo University Forest at Chichibu were assumed by the survey of the stumps in cut-over area. The results showed that about 21 per cent of the natural standing trees of these two species of *Tsuga* in this area were damaged by the butt-rots. Comparing with the data on the butt-rots of *Abies sachalinensis* and *Picea jezoensis* in Hokkaido, and of *Abies firma* in this area, these trees were rather durable for the attack of butt-rot fungi. *Fomitopsis annosa*, *Poria subacida* and *Ganoderma tsugae* were the main fungi causing butt-rots in these two species of *Tsuga*.

126. A detecting method of heart rot in standing tree by boring test. Haruyoshi SAHO (Misc. Inform. Tokyo Univ. Forests, 13, 19–26, 1960)

To detect a heart rot in a standing tree, a boring-test instrument, which enables the measurement of specific torque-energy of boring, was used. As a sample tree, *Larix leptolepis* decayed by *Phaeolus Schweinitzii* in the heartwood was tested.

The results of the experiment showed clear correspondence between actual rotted-wood in the heartwood and estimated rotted-portion by the measurement of specific torque-energy. However, very early stage of rot which showed faint discoloration could not be detected by this instrument.

127. Insect damage. Masatoshi NITTO (Rept. Sci. Invest. For. Wind-damaged in 1954, Hokkaido, Jap. 176–230, 1959)

This report is the summarization of the survey on insect infestation in wind damaged forests by the Typhoon Marie which visited Hokkaido in September, 1954. Two surveys have been made ; one covers 22 days from September 16 to October 7, 1955 and the other 18 days from August 22 to September 8, 1956.

In 1955, insects propagated mostly in wind damaged trees and no damage was found in standing trees.

Damages on standing trees in 1956 were found in the wind damaged stand where the temperature was comparatively high and where fallen trees were infested with high percentage in the previous year.

Of insects attacking dead standing *Picea jezoensis*, the dominant species was *Ips typographus* and then, the propagation of *Polygraphus* spp. was significant. Dead standing trees were mostly caused of boring of the adults of wintered *Ips typographus* and the attack of their first generation larvae and its distribution was few at the lower part of the stem and even on the rest of the whole stem. The fact that despite the large number of boring there was little emergence in trees, was due to the resistances of over population of boring, low temperature and much rainfall in summer and natural enemies.

In *Abies sachalinensis*, the attack of *Polygraphus proximus* and *Monochamus* sp. was severe.

The survey was made on the propagation of insects in the cleared area of wind damaged *Picea jezoensis* and it was concluded that the noxious insect, *Ips typographus* propagated in remained logs, fallen logs and slash and at the base of broken down trees the secondary insects propagated.

In the wind damaged area of mixed forests of conifer and broad leaved trees in the southern district, few insects propagated in wind damaged trees in the following year.

128. **Species composition of swarms of spruce bark- and wood-inhabiting beetles and their seasonal change.** Chikao NISHIGUCHI (Journ. Jap. Forestry Soc. 41, 270–274, 1959)

The writer analysed the structures of swarm of bark- and wood-inhabiting beetles attracted to poisoned trap logs of Norway spruce, *Picea Abies*, settled in 1957 and 1958 in the Tokyo University Forest, Hokkaido. In the first swarming period (mid April-mid May), during which the air temperature in the daytimes rose often above 15°C to 20°C, the swarm was composed of *Hylurgops* sp. (mainly *transbaicalicus*), *Xyloterus lineatus* and *Blastophagus piniperda*. In the second period (late May-early June), during which the air temperature exceeded 20° or 23°C frequently, the swarm was composed of many species of bark beetles and represented by *Ips typographus* and *Hylurgops inouyei*. In the third period (mid June-early July), *Tetropium castaneum* and *Dryocoetes* spp. (*autographus* and *hectographus*), in the fourth period (mid July-late July), *Monochamus urssovii* emerged dominantly and most species of bark beetles had finished swarming. In August only *Coelosternus* sp. emerged abundantly.

129. **Die Verteilungsformen der Rindenbrütern an den Kiefern im Hokkaido Forst der Universität Tokyo.** Chikao NISHIGUCHI (Misc. Inform. Tokyo Univ. Forests, 13, 27–38, 1960)

Um die Arten der Borken-, Bockkäfer, Rußler und sowie ihre Verteilungsformen an der Kiefernstämmen zu bestimmen, wurden die Rindenbrütern im Hokkaido Forst der Universität Tokyo in den Jahren 1956 bis 1958 untersucht.

Von der Nachforschung drei dominanter Arten an den unteren, mittleren und oberen Teilen der Stämmen, wurden vier Grundformen an der europäischen Kiefer und eine Form an der koreanisch-und Weymouthkiefer unterschieden.

Nachdem die europäische Kiefern mehr oder weniger von Kiefernschütte befallen oder von Erdmäusen geknabbert wurden, geben *Blastphagus piniperda* und *Pissodes nitidus* die schwerigsten Beschädigungen den Stammen dieser Kiefern.

Pityogenes chalcographus, *P. seirindensis*, *Cryphalus piceus*, und sowie *Ips typographus* sind die gewöhnliche Fichtenborkenkäfern. *Cryphalus* sind auch die gewöhnliche Fichtenborkenkäfern, und *Cryphalus laricis* ist Lärchenborkenkäfer. Trotzdem, ist es nicht selten,

daß sie auf die Stämme der verschiedenen Arten von Kiefer bei ihrer aktiven Vermehrung sich sammeln. *Ips cembrae* ist eine bemerkenswürdige Art von Kiefernbeschädigung.

130. **Synökologische Beobachtungen über Befall der stehenden Fichte durch Borkenkäfer.** Chikao NISHIGUCHI (Journ. Jap. Forestry Soc. 42, 64–73, 1960)

Dieser Bericht handelt von Verteilungen der als Dominant vorkommenden Borkenkäfer an den Stämmen der Fichten, Befallstypen, Reihenfolge der Angriffe und Verfärbungs- oder Abfallformen der Nadeln von befallenen Bäumen. Die Beobachtungen wurden während der Jahren 1956-1958 im Fichtenbestand (ca. 50 jährig) im Hokkaido Forst der Universität Tokyo durchgeführt.

Eine Anzahl ber Borken–, Bock– und Rüsselkäfer wurde an den befallenen stehenden Stämmen gefunden. Von ihnen trat folgende vier Arten als Dominant auf: *Ips typographus*, *Cryphalus piceus*, *Pityogenes seirindensis* und *Polygraphus jezoensis*. Wenn der Befallstypus der stehenden Fichte durch Borkenkäfer mit als Dominant auftretenden Arten kennzeichnet wird, wurden in unserem Bestand folgende Typen beobachtet: *typographus*-typus, *piceus*-typus, *seirindensis*-typus und Komplex-typus. Angriff wurde gewöhnlich mit *Ips typographus* begonnen. *Cryphalus*- und *Pityogenes*-angriff gleichzeitig mit oder sofort nach *Typographus*. *Polygraphus* trat als Nachzügler der oben genannten Arten auf. Bei Sommerbefall vergesellschaftete *Monochamus urssovii* oft mit ihnen. Verfärbung oder Abfall der Nadeln in der Krone wurde in vier Formen beobachtet.

131. **Test of an automatic nest-recorder.** Tomoo ROYAMA (Brit. Birds, 52, 295–302, 1959)

An automatic nest-recorder was designed for the purpose of recording the visiting activities of male and female birds separately. A test was made at a nest-box occupied by a pair of Great Tits (*Parus major*) in the Tokyo University Forest in Fuji, Yamanashi Pref..

The data obtained for the last five days of the fledging period were analysed to illustrate the uses of the apparatus in recording visiting frequencies, daily rhythms, the durations of periods at the nest, the time of the first and last vists each day, and the hours when the young left.

132. **The theory and practice of line transects in animal ecology by means of visual and auditory recognition.** Tomoo ROYAMA (Misc. Rept. Yamashina's Inst. Ornith. Zool. 2-14, 1–17, 1960)

The problems of line transects in animal ecology were dealt with. Several methods of the uses of line transects were compared with one another and criticised both from the theoretical and practical point of view.

The density of a certain woodland bird population was estimated by census and by sample counts. And by introducing the mathematical formulae which give a theoretical value of the density, the uses of the methods of the sample counts were discussed in detail.

133. **Water relation of root-pruned *Cryptomeria* seedlings.** Akira WATANABE (Misc. Inform. Tokyo Univ. Forests, 13, 39–45, 1960)

When roots of *Cryptomeria* seedlings were pruned in the autumn, transpiration rate decreased markedly and osmotic value increased fairly, but water content was not so much affected. As new roots developed, these values seemed to be approaching the normal level of the intact seedlings, but the differences between them of osmotic value and watr content increased again from the middle of October to the beginning of the dormant season. These phenomena were supposed to be due to the hardening of the seedlings. Root-pruning was

also very effective in preventing seedlings from the overgrowth in the autumn, which is one of the causes of the winter injury.

134. **Germination and hard seed of *Acacia mollissima* Willd. in relation to the date of collecting the seeds.** Izumi Ishikawa (Misc. Inform. Tokyo Univ. Forests, 13, 46–56, 1960)

When the color of legume turned yellow, the seeds, even not fully ripen, germinated as sufficiently as fully ripen seeds, so we can collect seeds for a fairly long time. It is very important in Japan where ripening season of the seeds of *Acacia mollissima* coincide with rainy season. Seeds dried slowly in shade develop hard seeds slowly, while those dried quickly in the sun develop hard seeds suddenly. When seeds were collected early and kept in refrigerator, they were prevented from developing hard seeds.

135. **An instance of root system survey applied with Saran screen.** Sukenaka Watanabe and Isao Tange (Misc. Inform. Tokyo Univ. Forests, 13, 57–61, 1960)

Root development of transplanted seedlings in the nursery is easily studied without changing the shape of root systems by means of burying plastic screens in various depth in the nursery soils before planting. Screen of 8 mesh and 3,000 denier showed the best results. An excavating use of pressured water of less than 0.5 atm. is favorable. In case of densely planted seedlings of *Cryptomeria japonica* of 120 cm high and an area of 180× 180 cm, it took 2 hours for sandy soil and 5 hours for loamy soil with 4 men to excavate entire root systems.

136. **Tests of fertilization technique on forest tree seedlings. Part I. Influence of the depth of fertilizer placement in soil on the growth of sugi-seedlings** (*Cryptomeria japonica* D. Don) Sukenaka Watanabe, Isao Tange and Yoshisuke Kasuya (Misc. Inform. Tokyo Univ. Forests, 13, 62–69, 1960)

Growth, root development, and absorption of fertilizer were best when fertilizers were given on soil surface, as compared with the case of placing fertilizer at 15 cm and 30 cm of depth.

137. **A formula expressing relation between rainfall and corresponding runoff.** Sadao Ogihara (Journ. Jap. Forestry Soc. 42, 106–109, 1960)

In 1958 the author presented a formula

$$y^3+ay-x^3=0$$

where x=rainfall, y=runoff, a=constant.

This formula had the following characteristics;

(1) simplicity of the form having only one constant,

(2) reasonability of the graph taking concave upward shape and never allowing y to exceed x. However, it still had the weak points (1) when $x\to\infty$, $y\to x$ and (2) it has an inflection point of which it is difficult to explain the reason of the existence.

Now, efforts have been made to supplement the above drawbacks and found a better formula as

$$y^3+3axy-x^3=0$$

138. **Hydrologic characteristics of Higashiyama Watershed with special reference to channel precipitation runoff.** Yoshinori Tsukamoto (Misc. Inform. Tokyo Univ. Forests, 13, 99–106, 1960)

In this study we firstly take storms in which surface or interflow is hardly observed.

Then, separate ground water runoff from storm hydrograph. Thus, we obtain component hydrograph due to only channel precipitation.

Channel precipitation P_{ch}, channel runoff Q and the change of channel storage ΔS during a small unit time interval are related to one another as

$$Q = P_{ch} + \Delta S \quad \text{.......................................(1)}$$

Put Q_1=initial runoff,
Q_2=final runff,
S_1=initial storage,
S_2=final storage.

Then, the above equation becomes

$$\frac{Q_1+Q_2}{2} = P_{ch} + S_1 - S_2$$

or

$$\left(S_1 - \frac{Q_1}{2}\right) + P_{ch} = \left(S_2 + \frac{Q_2}{2}\right) \quad \text{...................(2)}$$

From the decreasing curve of a proper component hydrograph the relation between Q and S can be deduced. When Q-S relation and P_{ch}-T (time) relation are given, hydrographs due to channel precipitation alone can be traced with the aid of equation (2). Comparing hydrographs thus obtained with ordinary hydrographs the runoff due to interflow or interflow plus surface flow can be estimated.

Applying the above method to gauging data obtained in Higashiyama Watershed in the Tokyo University Forest in Aichi Prefecture, where surface flow is hardly seen, the writer found;

(1) storm hydrographs caused by rainfalls less than 10 mm are due mainly to channel-precipitation runoff, and

(2) in rainfalls less than 30 mm in summer season channel-precipitation runoff and interflow have a linear relationship.

VII. Forest Products

Abstracts Nos. 139—146.

139. Studies on surface roughness of wood. III. Shape of dents on the surface of wood. Yôji KIKATA and Isao HIRAMATSU (Journ. Jap. Wood Research Soc. 5, 138–143, 1959)

It is necessary for measurement and indication of roughness of the surface of wood to know the shape of dents on the surface.

The results of the measurement of such dents in our investigation by the optical slit method are indicated in the tables.

1) Roughness due to wood structure:

The surface of wood has a substantial primary roughness due to wood structure. Shapes of dents, viz. vessels of Yachidamo (*Fraxinus mandshurica* RUPR. var. *japonica* MAX.), Lauan (*Shorea* sp.) and Buna (*Fagus crenata* BL.), and tracheids of Sugi (*Cryptomeria japonica* D. DON) were measured on the hand-work-planed surfaces.

2) Roughness of sawn surface by various circular saws:

Tooth set of saw dominates the surface roughness, and in the case of spring set saw

the opening width of the large roughness coincides with the sawn length of one round of the saw. But in Yachidamo it is not clear owing to intense raised fibers. The opening ratio and the opening angle remain constant regardless of feed speed. No-set saw and planer saw have few oscillations by smaller side clearance and the opening width of the large roughness corresponds with the sawn length of 1/4 round of the saw.

The small roughness of the surface develops by each tooth cut, but the tooth cut is disturbed by ray tissues of wood, so it becomes discontinuous. The small roughness corresponds with each 2–3 teeth cut, and the opening width, the opening ratio and the opening angle remain constant regardless of feed speed or kind of saw, being affected by irregularities of set and feed and due to wood structure.

140. Shearing moduli of plywood, hardboard and particle board. Shitomo MAMADA, Junya YAMADA and Shinji HIRAI (Journ. Jap. Wood Research Soc. 5, 127–130, 1959)

This study describes how the plate shear test shall be applied in measuring the shearing moduli of plywood, hardboard and particle board. A series of experiments were conducted with square plates loaded at corners just as the ASTM standard test procedure to check the accuracy of the theory of isotropic plates.

An experimental arrangement was prepared as shown in Fig., to apply four equal loads at the corners of a square plate, two loads at the extremity of one diagonal acting downward, and two at the extremity of the other diagonal acting upward.

From the theory of isotropic plate, the following equation is introduced, in which w is the deflection at the point of the distance u from the center on the diagonal,

$$w=\frac{3Pu^2}{2Gh^3}$$

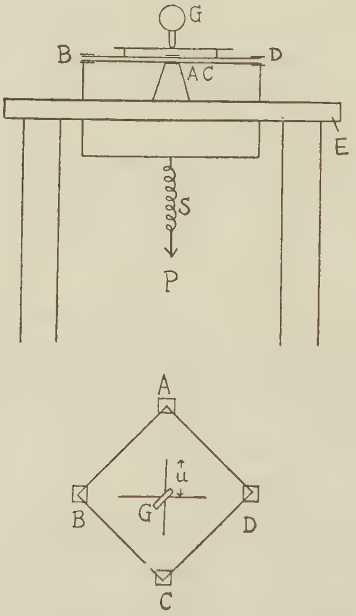


Fig. Experimental arrangement.

where, P is the total load applied to corners, h is the thickness of the plate, and G is the shearing modulus.
It follows that

$$G=\frac{3u^2P}{2h^3w}$$

The physical properties and Young's moduli of test boards are shown in Table.

The test results indicate that the values of shearing moduli are calculated from the equation affected by the length-thickness ratio a/h. It might be reasonable to make specimens have values of the ratio within 20–50. Generally speaking, this measuring and calculating method of shearing modulus might give satisfactory results for hardboard and particle board, but there are several disturbing factors inherent in plywood such as ply-construction, quality of veneer and the glue used.

Table: Physical properties and Young's moduli of test specimens.

	Thickness (cm)	Young's modulus (10^4·kg/cm^2)	Specific gravity*	Moisture content (%)
Plywood (3 ply)	0.36	9.1	0.65	10
	0.45	8.0	0.62	11
	0.58	8.1	0.63	10
	0.23	7.6	0.59	11
Hard board (standard)	0.35	4.0	0.93	6
	0.53	4.3	0.97	6
	0.65	5.4	0.99	6
Hard board (tempered)	0.35	5.0	0.98	6
	0.50	5.3	1.02	6
	0.65	6.2	1.04	6
Particle board	0.50	3.0	0.68	6
	0.75	3.8	0.70	6
	1.00	4.1	0.71	6
	1.25	4.2	0.71	6

* Based on weight and volume in air dry.

141. Adhesive properties of chemically treated plywood against powder-post beetle attack. Shinji HIRAI and Eiko AIZAWA (Wood Industry, 15, 246–248, 1960)

This paper is a part of report on preservative treatment of plywood against powder-post beetle attack, and deals with the abhesive properties of chemically treated plywood. Green veneers of white lauan (*Pentacme contorta* ROLFE) were spread with two kinds of preservative liquid, i. e. 1 : 1.5 mixture of boric and borax, and sodium fluoride of several different concentrations, piled up for several different times, and then glued with several kinds of adhesives, which were urea-resin adhesives extended by wheat meal and water, and soybean glue.

Specific gravity in oven-dry, moisture content, bonding strength in normal state, bonding strength after immersion in hot water of 63±3°C, delamination of glue line after immersion in water of room temperature and drying were examined. As a conclusion, there is no evidence of lowering adhesive properties of plywood by these preservative treatments of veneer.

142. On moisture proof of wood by paint film. Kinya TSUCHIYA and Kakuichi KITAHARA (Wood Industry, 15, 78–82, 1960)

The moisture absorption curve is very irregular and the value is lower when the test piece is taken out from the apparatus to be balanced the weight. And the authors prepared a special apparatus which can balance the weight without taking out the test piece from it. In general, capsule method is used for moisture absorption test of paint, but the authors tried to obtain results, which is combined directly to wood, so tested materials were prepared with paints and the wood of Hinoki (*Chamaecyparis obtusa* ENDL.). The paints used were lacquer, spar varnish and shellac varnish. The results obtained are as follows:

(1) The time to reach equilibrium condition in air becomes longer with the number of coat of paint as shown in Fig.

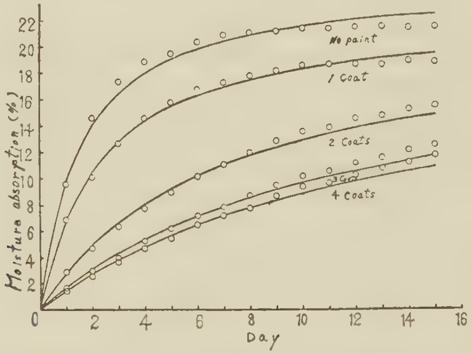


Fig. Relations of shellac vanish between moisture absorption and time.

(2) The efficiency of moisture proof of paint film is better with thicker paint film.

(3) The moisture proof of paint film is better with many coats when the thickness is similar.

(4) The efficiency of moisture proof of paint is better with more quantity and thickness of paint.

143. On the preparation of fiberboard by wet process applying urea-resin. Kakuichi KITAHARA and Akira NAGAHASHI (Journ. Jap. Wood Research Soc. 5, 201–207, 1959)

Phenol-resin is commonly used fiberboard to improve its physical and mechanical properties. But it is more expensive than urea-resin in Japan, consequently it is very desirable that urea-resin be used to better effect. The authors have studied the problem and the results obtained are as follows:

(1) The resin retention increases with added urea-resin as shown in Table.

Resin, added $\left(\frac{R}{W_o} \times 100\%\right)$	Retention $\left(\frac{R_r}{W_o} \times 100\%\right)$	Ratio of retention $\left(\frac{R_r}{W_o} \times \frac{W_o}{R} \times 100\%\right)$
2	0.54	27
5	0.73	15
8	0.90	11

R: Resin added (mg), R_r: Resin retention (mg), W: Pulp in oven dry (mg).

(2) The relation between Young's modulus and resin retention has a rising curve.

(3) Modulus of rupture increases with retention when the pressing temperature is low, but has a peak at 5% retention when the temperature is high. It indicates that heat destroys urea-resin.

(4) Brinell hardness increases with resin retention. It is large when the preessing temperature is high, but the effect of pressing time can not be determined in this experiment.

(5) Absorbed energy in bending test has a peak at 2% resin retention when pressing temperature is 100°C and 140°C. But it has the contrary tendency at 220°C, and the authors have been unable to find out the cause.

In short, urea-resin is effective in increasing Young's modulus and modulus of rupture, but the effect is little when pressing temperature is high, for urea-resin is destroyed by heat.

144. Fine structure of native cellulose fibers. Part 2. Difference between fine structure of wood pulp and linters pulp. Hiroshi NISHIMURA, Osakazu NAKAO and Nobuhiko MIGITA (Journ. Soc. Textile and Cellulose Ind. Jap. **16**, 289–295, 1960)

In order to compare fine structure of transitional area, an acetate grade cotton linter pulp and a viscose grade prehydrolyzed pine sulfate pulp were steeped in 4–18% sodium hydroxide solutions at 20°C, washed and dried, and then subjected to acid hydrolysis. Lateral order distribution curves showing the fine structure of transitional area in total, and each in the direction of length and width of micells (highly ordered regions) were obtained from the change in the amount of micells (known as crystallinity by hydrolysis method), leveling off degree of polymerization, and rate constant of hydrolysis, respectively. X-ray diffraction intensity curves of alkali-treated pulps were obtained as auxiliary informations.

Wood pulp has large amount of transitional area over wide range of lateral order in both dirctions of micells, while cotton linter pulp has smaller in the direction of length and scarcely in the direction of width of micells. From the results of X-ray diffraction measurements, it might be possible that wood pulp has some amount of micells which have especially small crystalline core, being decrystallized with sodium hydroxide solutions under 9%, while cotton linter pulp has no such fine structure.

X-ray measurements showed the presence of small amount of cellulose II lattice in the commercial pulps, and they increased gradually with increased concentration of steeping sodium hydroxide solution from 4 to 8–9%. These may be formed from relatively highly ordered regions due to rearrangement effect of swelling and deswelling.

145. Studies on water-borne wood preservatives. Part 13. Synthesis of fixtan acids, their derivatives and their critical micell concentration in aqueous solution. Takeo SHIBAMOTO and Yoshiyuki INOUE (Journ. Jap. Wood Research Soc. 6, 11–15, 1960)

Solubilization is the term given to a particular mode of bringing into solution substances that are otherwise insoluble in a given medium. We attempt to introduce this solubilization to the field of wood preservatives. The dissolution of water-insoluble organic preservatives in the surfactant solution has not been reported. Fixtan acids, most popular solubilizer, contain at least two sulfonated aromatic rings joined by an aliphatic bridge. The fixtan molecule picks up another molecule and forms a colloidal micell in water solution. If solubilizing ability is lost after preserving treatment, the solubilizate will be fixed strongly in wood.

We synthesized fixtan acids and their mercuric derivatives and measured their critical micell concentration (CMC) in aqueous solution.

The method and result obtained are as follows:

1) Fixtan acids were obtained by the reaction of naphthalens-, phenol- or *o*-cresol sulfonic acid and formaline. Fixtan acids were analyzed by elementary analysis, and infrared absorption spectral measurement. Mercury contained in derivatives of fixtan acids were determined by dithizone method.

2) CMC was determined by the dye absorption method using pinacyanol chloride, viscosity method and surface tension method. The viscosity was measured with an Ostwald-type viscometer at 15±0.05°C and the surface tension was measured with DU NOUY's apparatus at 20±0.5°C. CMC values of sodium or ammonium salt of three fixtan acids were $0.7-3.10^{-4}$ moles/*l*.

Part 14. Solubilized wood preservatives. Takeo SHIBAMOTO and Yoshiyuki INOUE (Journ. Jap. Wood Research Soc. 6, 29–33, 1960)

In recent years, solubilization is applied for cleansing, dyeing, medical application, agriculture and othr individual uses. But, we have very little information as to the application in the fields of wood preservatives. The solubilized preservative may be fixed more strongly without leaching out by water than common water-soluble preservative, and it may have better penetrability into wood than emulsifisable or oil-borne preservative.

This paper deals with the dissolution of water-insoluble organic preservatives in the surfactant solution.

The results obtained and discussions made are as follows:

1) 4,6-dinitro-*o*-cresol, pentachlorophenol, copper pentachlorophenate and dieldrin can be made to solubilize by the use of polyoxyethylene alkylamine.

2) Sodium alkyl sulfate, soapless soap, salt of fatty acid and numerous miscellaneous surfactant were not effective for solubilization of these preservatives.

3) These solubilized systems are more stable than emulsion and are viscous solutions which do not precipitate or emulsificate even if the solution be diluted with water to a concentration far less than the CMC. The dissolution mechanism is not yet clarified. It is presumed that the preservative is made hydrophilic by the oriented adsorption of the surfactant ions and behaves as if it were polyelectrolyte.

4) Decaying test of solubilized preservatives was tested by the method of JIS A 9302. These preservatives show good preserving effectiveness after 10 times weathering procedure.

Part 15. Phenyl mercury fixtan. (1). Takeo SHIBAMOTO and Yoshiyuki INOUE (Journ. Jap. Wood Research Soc. 6, 49–53, 1960)

In recent years, increasing interest has been shown in phenyl mercurial compounds as wood preservatives. But, their main drawback for actual use is their low solubility in water. It is reported that phenylmercuric 2,2'-dinaphthylmethane-3, 3'-disulfonate goes readily into colloidal solution by the use of 2,2'-dinaphthylmethane-3, 3'-disulfonate, and the chief components of phenyl mercury fixtan (PMF) are these two compounds.

This paper dealt with penetrability on filter paper, leachability and volatility from wood block and absorption on cellulose of PMF as a water-borne wood preservative.

The method employed and results obtained are as follows:

1) PMF was prepared by mixture of phenylmercuric 2, 2'-dinaphthylmethane-3, 3'-disulfonate and sodium 2, 2'-dinaphthylmethane-3, 3'-disulfonate (10.35:89.65) which were synthesized in the manner described in a previous paper. The desired concentration solution of the mixture was prepared. PMF has maximum absorption bands in the ultraviolet region at 265 and 290 mμ in water solution and no absorption in the visible region.

2) Penetrability of PMF solution on filter paper was compared with coefficient P (h^2/t),

where h is penetration length (cm) and t is time (10 minutes). The amount of mercury in filter paper had decreased gradually towards the top part of the filter paper.

3) For the determination of mercury contained in PMF, sample (treated wood 2×2×1 cm or filter paper 2×2 cm) was placed in a long-necked (25 cm) 50 cc Kjeldahl flask, and 5 cc concentrated sulfuric acid and 0.7 g potassium sulfate were added. Condensor was connected to keep the mercury from volatilizing. Then, the flask was heated gradually, and after being cooled, about 5–15 drops hydrogen peroxide was added. This decomposition procedure was repeated several times until the solution became clear. The error of mercury contained in treated wood is about 1% (0.1–0.5% solution).

4) Absorption on cellulose of mercury which was contained in PMF solution was observed, but this cannot be explained as the reaction between cellulose and PMF.

5) The leachability and volatility of PMF are considerably less than those of sodium fluoride by JIS A 9302. The former has better fixing ability than the latter. The rate of leaching of mercury from wood block treated with PMF solution is about 50% after 10 times weathering procedure.

Part 16. Phenyl mercury fixtan. (2). Takeo SHIBAMOTO and Yoshiyuki INOUE (Journ. Jap. Wood Research Soc. 6, 53–58, 1960)

In a previous paper, the penetrability on filter paper, leachability and volatility from wood block and sbsorption on cellulose of PMF (phenyl mercury fixtan) was described. In the present report, fungistatic activities of PMF and its mixtures are examined.

The method employed and results obtained are as follows:

1) The inhibitory effect of PMF by the poisoned agar method and cup method against *Poria vaporaria* was rather greater at neutral or acid than alkaline medium. Its effect was smaller than 4,6-dinitro-*o*-cresol in the same concentration.

2) Prevention of mold growth on bamboo stem needs 5 minutes steeping at 0.3% PMF solution.

3) 0.3% PMF solution shows good preserving effect in the decaying test by the method of JIS A 9302 after 10 times weathering procedure.

4) Small amount of dieldrin was solubilized and prepared as preliminary preservative containing PMF. Its insecticidal effect was rather little for preliminary preservation; therefore, it may be suggested that the amount of dieldrin was too small in this test.

Part 17. Color change of treated wood by weathering procedure. Takeo SHIBAMOTO, Yoshiyuki INOUE and Yoshihisa KENJO (Journ. Jap. Wood Research Soc. 6, 122–128, 1960)

In general, color change of treated wood caused by weathering is related to the formation of complex salt by reduced action of wood, preserving effectiveness, and paintability. We studied the color change of treated test blocks caused by weathering procedure. Test blocks used were Japanese cedar and bamboo which were treated with 14 kinds of preservatives. Spectral reflection was measured by spectro-photometer and tristimulus values and trichromatic coefficients were calculated.

The method and results obtained are as follows:

1) Intensity of ultraviolet spectra was measured by aceton-methylene blue method. Mercury lamp and sunlight have a very much greater intensity than biotron and black light illumination.

2) The color of wood changes by the condition of annual ring or figured grain, therefore, characteristic of its own color is hardly measurable.

3) The color of bamboo treated with *p*-nitrophenol solution was changed deeply by

illumination, but in the case of copper pentachlorophenate, treated wood was changed lightly by leaching or illumination.

4) Comparing the color of treated wood with Munsell color standard, great difference of lightness was observed.

5) The relation of color difference with lightness, dominant wavelength and excitation purity was investigated.

Part 18. Color change of wood treated with colorants caused by weathering. Takeo SHIBAMOTO, Yoshiyuki INOUE and Yoshihisa KENJO (Journ. Jap. Wood Research Soc. 6, 147–151, 1960)

In a previous paper, it was shown that color change of treated wood is caused by weathering procedure. In the present paper, an experiment was carried out to make colored preservatives. The color change of wood treated with colorants was studied. The method employed was essentially the same as in the previous paper.

From the results of experiments it was deduced that the colorants used are not adequate under severe weathering conditions, but some of them may be usable for moderate weathering. Further experiment is necessary for the making of colored preservatives.

146. Phenol oxidase in the wood-rotting fungi. II. Optimum pH with hydroquinone and oxidation of p-hydroxyphenyl-pyruvic acid by enzyme of *Poria subacida*. Toshio FUKUZUMI (Journ. Jap. Wood Research Soc. 5, 222–229, 1959)

1. The wood-rotting fungus, white rot, *Poria subacida* grew well on a medium in which the lignin was the sole source of carbon.

2. Enzymes were obtained by saturation with ammonium sulfate of the culture filtrate and water extract of the mycelium of *Poria subacida*, which was cultivated on the medium containing lignin. The optimum pH of these enzymes was pH 2.0–3.0 for hydroquinone.

3. The above enzyme from the culture filtrate was separated to fraction A and fraction B. Fraction A was obtained by half saturation with ammonium sulfate, and fraction B was obtained by complete saturation.

Mixed enzyme of fraction A and fraction B oxidases p-hydroxy-m-methoxyphnylpyruvic acid, consuming one molecule of oxygen per molecule of the substrate under liberation of carbon dioxide.

VIII. Zootechnical and Veterinary Science

Abstracts Nos. 147—163

147. Relation between dairy performance and mammary glands and their stimulators. III. Free and protein bound estrogen levels in the blood of goats treated with synthetic estrogen for induced lactation and their relationships with lactation results. Hiroshi NAGASAWA, Asahiko KOHNO, Yôichi SHÔDA and Motoo NAITO (Jap. Journ. Reprod. 5, 45–47, 1959)

This experiment has been carried out in order to examine the possibility of use of Euvestin sol (diethylstilbene+diethylstilbestrol) instead of Euvestin oil (diethylstilbene) for the convenience of practical application in the induction of lactation, by investigating the levels of estrogen in the blood of goats treated with Euvestin sol or oil, and the relation

between their levels and lactation results induced by Euvestin injection.

Animals used were five virgin goats, and among them, three were injected intramuscularly with Euvestin sol and the others subcutaneously with Euvestin oil. Free and protein bound estrogen levels in the blood were measured by the mice intravaginal assay method. Milking was done once a day from the day of the first injection. As the lactation results, total milk yield, average daily yield, maximum period, duration of lactation and average butter fat percentage were measured.

The results obtained are summarized as follows:

(1) Free estrogen levels increased and maintained in comparatively high levels about from the 12th to the 15th day and afterwards decreased. Concerning to protein bound estrogen, no definite tendency was seen.

(2) In either Euvestin sol or oil group, no relation was found between results of induced lactation and estrogen levels in the blood.

(3) There was no difference in total milk yield and other characters, owing to the conditions and injection methods of Euvestin.

148. Establishment of mice strains with special reference to mammary growth response. IV. Selection for the mammary growth response through selection for the body weight in mice. Jiro NAGAI (Jap. Journ. Breed. 9, 82–88, 1959)

Although the mammary growth response in mice may be the index of the early selection for milk yield at lactation period, it takes much time and labor to obtain the value of the response. For the purpose of investigating a simple way of selection for the mammary growth response, the efficiency of selection for mammary growth response through selection for body weight every generation was compared with that of the direct selection for mammary growth response every third generation. The mice used were 627 of Kasukabe female mice. They were injected five times with total dose of 12.5 I. U. of estrogen, from 20 days of birth. On 35 days, the right third mammary glands were prepared to obtain mammary growth response after weighing.

Results obtained are as follows:

1. Intra-litter correlation, intra-dam correlation and heritability were 0.37, 0.12 and 0.51 in mammary growth response and 0.65, 0.26 and 0.55 in body weight.

2. In the case of selection for body weight, selection accuracy using a measure of body weight of a mouse is higher than that using average body weight of family which is composed of fullsister.

3. In the case of direct selection for mammary growth response, the use of the average mammary growth response of more than eight mice is more accurate than the use of a measure of mammary growth response of a mouse.

4. In consideration of selection interval, the selection for body weight is generally more efficient selection for mammary growth response compared with the direct selection for mammary growth response.

V. Difference of mammary duct area between intact and estrogen treated female mice. Jiro NAGAI (Endocrinol. Japon. 7, 34–38, 1960)

The mammary growth in female mice treated with estrogen was compared with that in intact female mice. Half of females produced from 18 parents were injected with a standard dose of estrogen and the other half were kept without any treatment until 35 days after birth.

Results obtained are as follows:

1. No difference was found in body weight between treated and intact female mice on both 20 days and 35 days after birth.

2. Difference of mammary duct area and uterine weight was found between two groups.

3. Variance of mammary duct area in treated mice seemed to be smaller than that in intact mice. The fact might be caused by the decrease of relative variation of amount of estrogen acting on the mammary gland.

149. The effects of prolaction on the respiratory activity of normally lactating and of unilaterally ligated mammary glands in the mouse. Hideo MIZUNO and Tateki CHIKAMUNE (Endocrinol. Japon. 6, 153–160, 1959)

Prolactin was injected to the normally lactating and unilaterally ligated mice in order to ameliorate or maintain the respiratory activity of the mammary gland. From the results of the histologial observation and litter's growth curve in addition to the respiratory activities, it was suggested that prolactin might exert some influence on metabolic or synthetic process, qualitatively but not quantitatively. And it was discussed that there might exist a certain optimum dose according to the condition of mammary gland for prolactin to exert stimulatory effect on the mammary respiratory activity. However, prolactin, in adopted doses, did not prevent the involution occurring in the ligated gland six days after unilateral ligature. From these results it may be possible to conclude that the removal of milk might be a limiting factor, and further, prolactin may by effective for maintaing the mammary gland in a functional state, although the dosage of hormone in connection with the stage of lactation is an important factor.

150. The effects of administration of prolactin, progesterone or concurrent pregnancy on the mammary gland involution and respiratory activity in the mouse. Hideo MIZUNO (Endocrinol. Japon. 7, 121–131, 1960)

The effects of administration of prolactin, progesterone of concurrent pregnancy on the mammary involution occurring in the ligated side and on lactational function in the unligated side were examined in the mouse undergoing the unilateral ligature of nipples.

The administration of 20 I. U. prolactin or 1 mg progesterone daily for 6 days could retard the involution in the sense that alveolar integrity is still preserved despite of the relatively small amount of parenchyma, whereas alveolar structure and cell boundaries could hardly be defined in the non-treated control.

Concurrent pregnancy retarded the involution markedly and section showed the essentially similar appearance to that at the latter part of pregnancy or shortly before parturition.

However, the suggestion that the removal of milk may be responsible for maintaining the mammary gland in a full lactational state was made from the results of histological observations and respiratory activity. And further the hormonal balance for mammary growth and lactation was discussed in connection with the removal of milk.

Progesterone alone did not inhibit the respiratory activity. The effect of pregnancy on lactation should be awaited further investigation in connection with the hormonal levels circulating in pregnant animal, which may vary according to the fetus numbers and its developmental stage in relation to delay of implantation.

151. Properties of the neuromuscular unit in respiratory muscles. Hiroshi SAWAZAKI (Jap. Journ. Vet. Sci. 21, 227–232, 1959)

The time series in the logarithmic scale of the discharge interval of a NMU in respiratory muscles are divided into several parts in compliance with the respiratory cycle. The mean

value ($\log \bar{z}$), and the standard deviation ($\log S$) are calculated in each part. Thus, the functional properties of the NMUs in respiratory muscles can be discussed in the same manner as the $\bar{z}$–S correlation in skeletal muscles.

Properties of the NMU in respiratory muscles of the dog were examined by means of the $\log \bar{z}$-$\log S$ correlation under the anesthetized condition, and in forced breathing after the deafferentation by the removal of the dorsal roots and ganglions on both sides in spiral segments at Th 9–12.

1) Three or four months after the operation, the distribution of the $\log \bar{z}$-$\log S$ points could be separated into two groups (the left group was designated as A, and the right group as B).

2) Under the anesthetized condition, the $\log \bar{z}$-$\log S$ points belonging to the A group moved from their own region to that of the B group.

3) The points belonging to the B group in quiet breathing, were displaced to the region of the A group by forced breathing, and these points returned to their own region with the release of the load. On the contrary, no response was observed in the attitude of the points belonging to the A group in this conditioning.

4) The distributions of both groups were clearly distinguished in all activity patterns.

From these results, it is presumed that the kinetic NMU and the tonic NMU are recognized in respiratory muscles as well as in skeletal muscles.

152. **On the maintenance of implanted embryos in the lactating rats.** Koji YOSHINAGA and Tosiro HOSI (Jap. Journ. Animal Reprod. 5, 87–90, 1959)

Prolongation of pregnancy period in the lactating rat is due to the delay in implantation of fertilized ova. But this fact does not mean that the lactation has no effect on the maintenance of embryos after implantation. This paper is concerned with the effect of lactation on the implanted embryos and the results obtained by the administration of ovarian hormones in maintaining the embryos in lactating rats.

I) Temporary reduction in the number of sucklings induced normal implantation in the lactating rats: Litter size of a pregnant lactating rat was reduced from 6 to 2 on 4th day of pregnancy. On 6th day, two days later, number of sucklings was increased from 2 to 8 with the same age pups and let them suckle to the end of experiment. Implantation at the normal time was confirmed by the laparotomy on 9th day of pregnancy.

Seven of 8 thus treated rats resulted in resorption of the implanted embryos, which was confirmed by laparotomy or resorption sign (appearance of fresh blood in vagina) from 9th to 12th day of pregnancy.

II) Daily injections of progesterone were started from 6th day of pregnancy by subcutaneous route to the rats under the same experimental conditions as experiment I. In the 2 mg per day group, all embryos were resorbed in 2 of 4 cases and in other cases pregnancies were maintained to result in parturition with small litter size. In the 5 mg group, on the other hand, in all 4 cases pregnancies were maintained to result in parturition with normal litter size.

III) Estrone was used in stead of progesterone under the same experimental conditions as with progesterone. Daily injections of estrone were made from 6th to 20th day of pregnancy. Daily $0.5\mu g$ of estrone maintained pregnancies in 4 of 5 cases, and $1.0\mu g$ maintained them in all 5 cases.

IV) For the induction of implantation in the lactating rat, temporary reduction in litter size was used in the above-mentioned experiments, but estrogen injection can also be utilized

for this purpose. In this part of experiment, therefore, 1.0 μ g of estrone was injected to rats suckling 6 pups on the 5th day of pregnancy for the induction of implantation and 2 same age pups were added at the same time. Necessary period of estrone administration daily 1.0 μ g in maintaining the embryos was determined in these rats. Implantation sites were counted on 9th day of pregnancy.

Four days' injections could not maintain the embryos, but injections more than 6 days maintained them and resulted in parturition : 6 days' injections maintained them in 1 out of 5 cases, 8 days' in 3 out of 6 and 10 days' in all the 4 respectively.

It is evident from this experiment that necessary period of estrone treatment in maintaining the embryos is 10 days, that is, until 14th day of pregnancy. This is coincident with the time when placental sign appears in a pregnant rat. Compared with the numbers of implantation sites on 9th day, the numbers of new born pups were fairly small. This fact suggests partial abortion had occurred during the experiment.

In these experiments it was observed as an outstanding phenomenon that increase in body weight of suckling litters stopped suddenly about when placental sign appeared in the mother that had escaped from the resorption by the hormone treatments.

153. **Release and replenishment of acetylcholine in intestinal wall of guinea pig.** Toyoaki HAYAMA and Miyoshi IKEDA (Amer. Jour. Physiol. **197**, 923-925, 1959)

The total acetylcholine (ACh) content of the guinea pig ileum decreased (10-20%) immediately after circumferential stretching. Morphine was found to inhibit the decrease in the ACh content, while smooth muscle stimulants (ACh, histamine and KCl) exhibited no remarkable effect on the process. After ceasing the streching the ACh content was restored within 15 seconds to the initial level. Successive peristalses also were found to result in a decrease in the ACh content of the intestinal wall. In relation to the circumferential stretching of the intestinal wall, a possible scheme of the sequence of reactions, comprising rapid release of ACh followed by decomposition and resynthesis, occuring during the course of stretching and relaxing of the intestine, is discussed.

154. **Factors necessary for *Eimeria tenella* infection of the chicken. VI. Excystation of oocyst in vitro.** Miyoshi IKEDA (Jap. Journ. Veter. Sci. 22, 27-41, 1960)

Based upon previous reports, the present experiments were undertaken in order to obtain further evidence, in detail, concerning the process of excystation in vitro of the oocysts of *Eimeria tenella* in mediums containing pancreatic enzymes, and to study an essential component of the pancreatic juice in order to bring about the liberation of the sporozoites from the oocysts and to determine the main site of its action on the oocysts.

The *E. tenella* oocysts used were collected from the cecal contents of chickens infected with the pure line of *E. tenella*, and observations on the excystation were made by employing the hanging drop preparations. The results obtained were as follows :

(1) The excystation of the oocysts of *E. tenella* was induced by the enzymatic action of the pancreatic preparations under suitable conditions in vitro. The liberation of sporozoites was accomplished by the active and spontaneous movements of the sporozoites themselves through both micropyles, initially of the sporocysts and then of the oocysts.

(2) The behaviors of the sporozoites, in the course of the excystation, were observed as follows :

At the beginning of incubation the active appearances of oocysts were usually seen, and then the sporozoites started moving within a sporocyst, at first very slowly and then becoming more active, and occasionally, corresponding with their active rotating movements, the whole

of the oocyst also moved slightly. In the meantime, the moving sporozoites came out through tne micropyle of the sporocyst and after having left the sporocyst, the freed sporozoites swam about within the oocyst, actively, as if seeking for an opening, and soon squeezed themselves through the region of the micropyle located at the narrow end of the oocyst wall, showing their peculiar behaviors in leaving the oocyst through this region in exactly the same manner as the micropyle of the sporocyst.

(3) As to the essential enzyme in the pancreatic juice which brings about excystation, experiments were undertaken by using the following enzyme solutions; the commercial pancreatic preparations, such as trypsin and pancreatin with a high tryptic activity, or crystalline trypsin, and the physiological saline extracts of pancreas (chicken), and the duodenal juice activated by enterokinase. In all cases, positive results were obtained by the exposure of the oocysts to such enzyme solutions, in regard to the effects on excystation. On the contrary, in the case of any enzyme preparation, such as a pancreatic preparation with a low tryptic activity, the enzyme other than trypsin in the pancreatic juice, crystalline chymotrypsin, and the commercial papain, if substituted for trypsin, no excystation could be detected in any of the experiments.

In order to know the effects of digestive enzymes other than the pancreatic juice on the excystation, observations were made by using enzyme solutions such as the various fluids collected from the crop, gizzard and propendiculus, and from the small intestine of a chicken 7 days after the ligation of its pancreatic ducts. In all cases no evidence of any excystation of oocysts could be observed.

In further experiments, even in the case of exposure of the oocysts to gastric digestion prior to the action of the trypsin, no significant influence on the excystation could be detected.

From these results, it was concluded that trypsin is the specific enzyme necessary for the excystation of oocysts in vitro. Besides it seemed probable that the process of excystation, as assumed by SMETANA (1933), is induced by the changes of the particular substance, probably a kind of protein substance sensitive to trypsin, closing the micropyle, and then, the active movements of the sporozoites themselves, stimulated by the influences of the media, allowing their escape through this region.

However, the results obtained, particularly the length of time required to bring about excystation of the oocysts in vitro, varied considerably, depending on the many factors concerned. In respect to these points, the possible causes were discussed.

VII. The infective forms of oocysts (*Eimeria tenella*). Miyoshi IKEDA (Jap. Journ. Veter. Sci. 22, 111–122, 1960).

In recent literature on chicken coccidiosis, it was reported that unsegmented, or, unsporulated oocysts, complete their sporogony in the crop or the gizzard and cause coccidiosis in the chicken. This is contrary to the generally accepted view that they must become segmented before becoming infective, under favorable natural conditions. Because of such discrepancies in regard to the infective forms of oocysts, the present experiments were undertaken to determine what form of the developmental stage of the oocyst can produce the infection of *Eimeria tenella*.

The oocyst materials used, were collected from the cecal contents of chicks infected with *E. tenella*, and then placed in 2.5% $K_2Cr_2O_7$ solution and stored at 6–7°C.

(1) In order to observe the time course of the developmental process of the oocysts, observations are made by using the hanging drop method in which a few fresh unsegmented oocysts were suspended in a drop of 2.5% $K_2Cr_2O_7$ solution and observed under the micro-

scope placed in an incubator at 27.5±0.5°C. In the course of sporogony the characteristic changes in the oocysts were observed through the followiog six stages, as described by NOMI (1932), including the time from the beginning of the incubation, to the time of the full development of the oocysts :—— stage of cytoplasm reduction, stage of nuclear division, stage of cytoplasm transformed, pyramidal stage, stage of sporoblast, and stage of sporozoite.

(2) Ingested unsegmented oocysts do not sporulate in the alimentary canal, owing to the unfavorable conditions (mainly temperature and oxygen) governing the maturation of the sporozoites.

(3) As to the question of whether or not unsegmented oocysts can cause infection, the groups of chicks were inoculated, respectively, with oocysts in the same amounts but in different stages of development, and at various intervals after incubation; the object being to determine the infective form of the oocysts, and the time required for the unsegmented oocysts to become infective after their incubation in $K_2Cr_2O_7$ 2.5% solution at 27.5°C. All the oocysts were inoculated by means of a syringe, directly into the crops of the chicks. The final conditions (the developmental stage) of the oocysts used in inoculating, were determined at each time of inoculation, by an estimation made from the results obtained by a smear method of detecting these oocysts. They were then compared with the six developmental stages classified above.

From the results obtained, it was confirmed that infection of *E. tenella* occurs only through the inoculation of sporulated oocysts (contained sporozoites), because no evidence of any infection occurs through the inoculation of unsegmented oocysts, or, even those in any other stage of development.

155. Some chemical and pharmacological properties of an amine (maltoxin) isolated from malt rootlet. Norimoto URAKAWA, Toyoaki HAYAMA, Takehiko DEGUCHI and Yoshio OHKUBO (Jap. Journ. Pharmacol. 9, 41–45, 1959)

Some of the chemical and pharmacological properties of an amine, maltoxin, isolated from brew malt rootlet were studied. The results obtained were as follows :

Maltoxin was extracted successively with ethanol and HCl followed by several steps of purification, and was finally obtained as a hydrochloride.

Qualitative analyses of maltoxin revealed that maltoxin might be an aromatic amine with or without phenolic OH.

Maltoxin elicited the contraction of the isolated rectus muscle of frog. The pharmacological response was applied for the bioassay of maltoxin.

Several amines known to be contained in the rootlet were found to exert, if any, only weak effects on the rectus muscle in comparison with maltoxin.

156. The paralyzing action of maltoxin, and active principle from malt rootlet, on the frog muscle. Norimoto URAKAWA, Takehiko DEGUCHI and Yoshio OHKUBO (Jap. Journ. Pharmacol. 9, 130–136, 1960)

Maltoxin, an amine obtained from brew malt rootlet, exhibited a paralyzing action on the intact frog. This effect was shown using Claude-Bernard's preparations and the nerve-sartorius preparations of the frog to be due to the block of the neuromuscular junction.

1×10^{-5} pure maltoxin blocked neuromuscular transmission completely about five minutes after application and also decreased the height of twitches of directly stimulated muscle to an extent of about fourty-five per cent in the nerve-sartorius preparation of the frog. In the continued presence of the drug in the bath recovery from the muscle depression was observed.

Relations of the concentration to the depression of the responses of directly and indirectly stimulated muscles and to the time needed for the complete neuromuscular block were presented.

The neuromuscular blocking action of maltoxin was discussed. Maltoxin differs from *d*-tubocurarine in that there is a period of excitation preceding the neuromuscular block in the nerve-sartorius preparation of the frog.

157. **Stabilization and rectification of muscle fiber membrane by tetrodotoxin.** Toshio NARAHASHI, Takehiko DEGUCHI, Norimoto URAKAWA and Yoshio OHKUBO (Amer. Journ. Physiol. 198, 934-938, 1960)

The mode of action of tetrodotoxin on the frog muscle fiber membrane has been analyzed with the aid of intracellular microelectrodes. Tetrodotoxin of 10^{-7} concentration made the applied cathodal current ineffective in producing action potential, whereas the resting potential and resting membrane resistance underwent little or no change. With 10^{-8} tetrodotoxin the muscle fibers responded with the small action potentials at high critical depolarizations. These results can be explained on the basis of the membrane being stabilized by inactivation of the sodium-carrying mechanism. Although delayed rectification was not observed in normal muscle fibers, it became apparent in the fibers rendered inexcitable by tetrodotoxin. This finding, together with other evidence in the existing literature, supports an applicability of the sodium theory to the frog muscle fibers.

158. **Dual action of maltoxin, a neuromuscular blocking agent from malt rootlet.** Norimoto URAKAWA, Toshio NARAHASHI, Takehiko DEGUCHI and Yoshio OHKUBO (Amer. Journ. Physiol. 198, 939-942, 1960)

Maltoxin, an amine obtained from brew malt rootlet, causes a neuromuscular block in the frog. Its mode of action on the frog's sartorius muscle has been studied mainly with the aid of intracellular microelectrodes. Maltoxin of 10^{-5} concentration caused an over-all depolarization of the muscle fibers ; therefore, direct and indirect stimulations of the muscle were ineffective in producing muscle action potential. Progressive repolarization took place thereafter and the muscle became excitable by direct stimulation, though indirect stimulation remained ineffective for a long period of time. Small end-plate potentials observed could not be explained in terms of depolarization. It was found that the sensitivity of the end-plate membrane to acetylcholine was effectively depressed by maltoxin, and that this is responsible for the persistent neuromuscular block. The initial over-all depolarization caused by maltoxin was fairly antagonized by a previous treatment with *d*-tubocurarine. It is concluded that maltoxin has a dual mode of action, having some resemblance to acetylcholine, decamethonium, succinylcholine and nicotine.

159. **Studies on microflora in the alimentary tract of livestock. I. Method for the culture of the bacteria in rumen.** Yuichi OCHI, Manabu OGATA, Tomotari MITSUOKA, Choji KANEUCHI and Hiroshi KAWAGUCHI (Jap. Journ. Vet. Sci. 22. 167-174, 1960)

The nutritional failures in livestock have become one of the most important problems in recent years, the importance of microorganisms in ruminant digestion has been generally accepted. Although a few studies on the rumen flora have been published, much confusion still exists.

The present work was undertaken to develop and prove a medium and technique suitable for the culturing of bacteria in the rumen. This is intended to form a basis for future works on rumen flora and their significance in the nutrition of the host.

Results obtained from this study were as follows:

1) Anaerobic incubation was essential for the culturing of rumen bacteria.

2) The medium which gave the best results was glucose-yeast-blood-liver agar, incubated anaerobically. This medium gave much better results for the kinds, number, and identification of rumen bacteria than reinforced clostridial medium (modified), nutrient agar, blood agar, rumen fluid glucose cellobiose agar, nutrient agar with rumen fluid, and blood agar with rumen fluid. It was also demonstrated that the streak plate culture method was suitable for the identification of bacteria and was of great convenience.

3) Although glucose-yeast-blood-liver agar plate was incubated by either phosphorus jar method or CO_2 jar method, no significant effects of CO_2 were shown.

4) Incubation period of 4 to 5 days was satisfactory for the culturing of rumen flora.

5) To detect cellulolytic organisms, rumen-fluid-cellulose medium incubated by CO_2 bubbling method was much better than the Omelianskii medium incubated aerobically.

160. On anemia of cattle with piroplasma infection (*Theileria* sp.) at the Asama pasture. Shin OKOSHI, Noritoshi KITANO, Isamu TOMODA, Manpei USUI, Mitsuo TAKASHIO, Naoyoshi SUZUKI and Mitsumasa WATANABE (Jap. Journ. Vet. Sci, 21-6, 70, 1959)

Cattle at pastures on the foot of Mt. Asama have been suffered from a heavy anemia, that has been called commonly "Yama-make" or "Mountain-illness". The authors investigated the disease of cattle at Tsumakoi and Asama pastures, and concluded that this anemia was caused by *Theileria* sp. and malnutrition due to poor grassland of the pastures. Treatment test was carried out.

The Asama pasture, that situates on a slope on the mountain 1,000 meters above the sea level, has poor grasses because of its volcanic ash soil. Cattle are put out to pasture there from June to October each year. They are exposed to a massive tick infestation during the term. A good part of the ticks are *Haemaphysalis bispinosa* and remaining of them *Ixodes japonensis*.

This disease occurs during July and August. The main symptoms are pyrexia, anemia, icterus, abortion, malnutrition and constipation. By the constipation the feces have a characteristic form resembling those of goat. The number of erythrocytes decreases down to 200 to 100 millions per 1 cubic mm. Hemoglobin index increases to a very high degree. *Theileria* sp., 0.4 to 3.9 micron in length, was found in many erythrocytes.

At the Asama pasture, the occurrence of the disease was 19.9% (or 28 head) and mortality was 25% (or 7 head) of total number of 141 head in 1957. In 1958, the occurrence was 23.3% (or 28 head). But all diseased cattle have been completely cured by oral administration of tropochin, intravenous injection of baberadin and the other cardiac and nutritional medicines.

The authors are convinced that the etiology of this disease may not be poisoning from some plants or minereal pollutions, nor simple malnutrition, but theileriasis with malnutrition.

161. Pharmacological studies of mikamycin on dogs. Shin OKOSHI, Noritoshi KITANO, Isamu TOMODA, Mampei USUI, Mitsuo TAKASHIO, Naoyoshi SUZUKI and Tomiji KONISHI (Journ. Antibiotics, Ser. A, 13, 137-142, 1960)

The antibiotic mikamycin was isolated from a broth culture of *Streptomyces mitakaensis* by ARAI *et al.*, of the Institute of Applied Microbiology, the University of Tokyo, in 1956. Thereafter, another antibiotic was found in the same broth culture and the previous one was named mikamycin A and the other mikamycin B. As described by TANAKA *et al.*, these antibiotics inhibit mainly the growth of gram-positive bacteria both *in vitro* and *in*

vivo and show no cross-resistance with those antibiotics which have been in medical and veterinary use up to date. Mikamycins A and B show a high synergistic action which becomes the most significant when 30 to 90 % mikamycin A is contained in the mixture of mikamycin A and mikamycin B. TANAKA *et al.* reported that *Staphyloccus aureus*, when treated with B, raised its sensitivity to A.

With mikamycins supplied by the Institute of Applied Microbiology, the authors studied absorption and excretion of the antibiotics in dogs and their toxicity to dogs in case of long-term oral administration. The results are as follows :

1. Three adult female dogs were administered orally with a dose of 100 mg/kg of mikamycin daily for 60 days.

2. None of them showed vomiting, diarrhea, or any other side-effect. Their clinical conditions, such as body temperature, respiration rate, pulse, and body weight, underwent no changes that might be considered as induced by the administration of mikamycin.

3. The results of blood examinations and liver and kidney function tests carried out on these dogs indicated no significant variations or changes that might be due to the administration of mikamycin.

4. Three dogs were killed for autopsy after the experiment and showed no pathological changes in their organs.

162. An outbreak of toxascariasis (*Toxascaris leonina*) of dogs in Tokyo. Shin OKOSHI, Noritoshi KITANO, Mitsuo TAKASHIO, Mampei USUI, Isamu TOMODA and Naoyoshi SUZUKI (Jap. Journ. Vet. Sci. 21-6, 63, 1959)

A species of dog ascarid, *Toxascaris leonina* (von LINSTOW, 1902), is a common inhabitant in the intestine of dogs, cats and other wild carnivores in U. S. A., U. S. S. R. and other foreign countries. Yet its occurrence has not been reported up to now in Japan, except in a few cases of imported dogs, and in some lions and tigers at zoological gardens.

In autumn of 1958, the rabies vaccination and parasitological examinations were carried out for a large number of dogs, which were kept for medical purpose at one place in Bunkyo-ku, Tokyo. It was then discovered that toxascariasis (*Toxascaris leonina*) was enzootic among them as follows :

At first the etiological parasite was identified as *Toxascaris leonina* (von LINSTOW, 1902) by careful inspection of worms and eggs obtained from a certain number of the dogs killed for autopsy. Then fecal examination was carried out for all the dogs remaining.

The dogs examined were 106 mongrels, aged presumedly one to 6 years, and of about 10 to 25 kg. of body weight. Seventy eight of them were kept in dog houses built on concrete floor and the remainings in those built on earth floor.

The infection (or egg positive in the feces) rate was 9.0% among the dogs kept on concrete floor and was higher as 32.1% among those kept on earth floor. The infection rate was 15.1% for all the dogs examined.

The infection rate varied in dogs according to the duration of keeping in the places. The rates were as follows ; 4.0% (or one head) among dogs kept for less than 2 months, and 32.0% (or 8 head) among those kept for 3 to 6 months. This fact suggests that the new commers may be infected with the parasite and the latter becomes to sexual maturity in the body of dogs after a certain period. And is more probable that the prepatent period of this parasite in the dog might be longer than 21 to 23 days or that of *Toxocara canis*, the far more common dog ascarid in Japan.

Usually the more common dog ascarid, *T. canis* infects rarely adult dogs of more than

one year old, however, the dogs involved in this enzootic were almost all adult dogs of more than one year old. The occurrence of *T. canis* among them was observed in two younger dogs, and one of them was infected mixedly with *T. canis* and *T. leonina*. The other was only with *T. canis*.

The female worms were 42 to 115 mm. in length and the male were 32 to 68 mm. The eggs were 63 to 81 μ in width with mean of 69 μ, and 77 to 101 μ in length with mean of 87 μ. The egg was of light color, with thick and smooth shell, and had a space around the ovum cell ball within the shell.

165. Studies on vitamin A deficiency in domestic animals. Part 1. Storage of vitamin A in the liver of dairy cattle in Japan with special reference to its seasonal fluctuation. Kazuya Usui, Tetsuo Fujita ane Kazuyuki Inoue (Jap. Journ. Vet. Sci. 22, 159–166, 1960)

The liver reserves of vitamin A in dairy cattle were measured over a period of 14 months beginning in January 1958.

Animals used for this study were 182 Holstein-Friesian cows which were killed at the slaughterhouse in Tokyo. The vitamin A contents in the liver and the mammary gland together with the plasma vitamin A and carotenoids concentrations were determined. The measurement of the glycogen levels in the liver was also performed.

The results obtained were as follows ;

1) The vitamin A contents in the liver were the lowest in April and the highest in December.

2) The reduction in the storage of vitamin A in the liver was brought about by the proliferation of the connective tissue.

3) The interrelationships between vitamin A and glycogen levels in the liver were not observed.

4) The vitamin A contents in the liver had any distinct correlation neither with plasma vitamin A levels nor the vitamin A contents in the mammary glands.

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